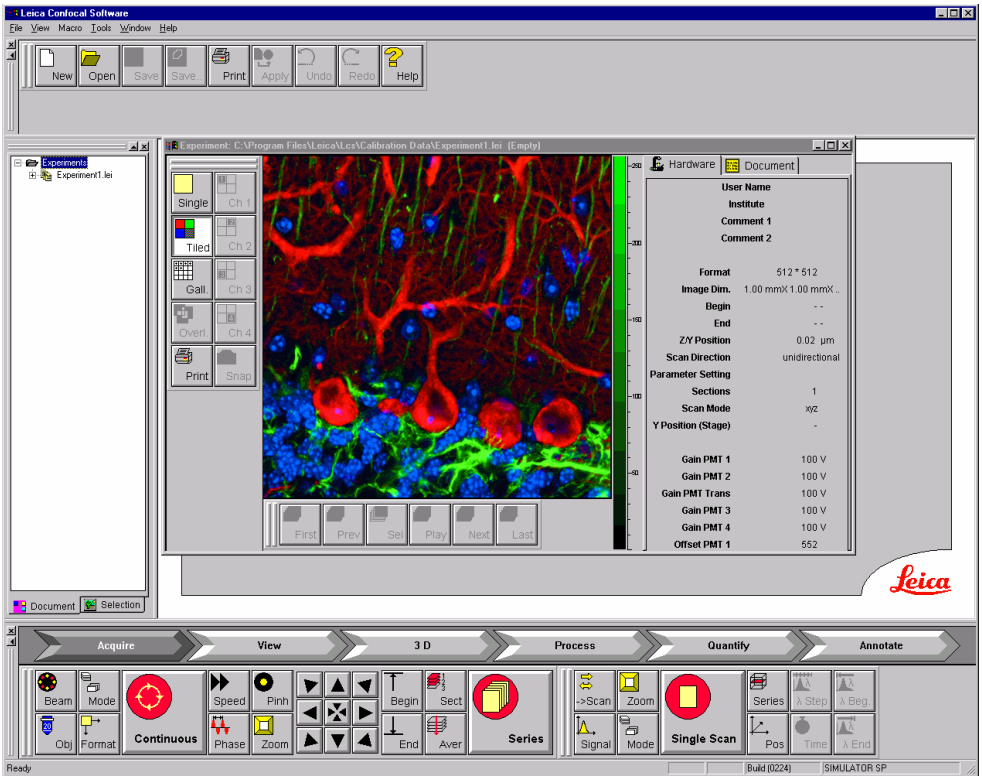

Leica Confocal Software
LCS

User Manual

Leica
MICROSYSTEMS

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Legal Notes

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This instrument is designed and manufactured to comply with applicable performance standards for Class 3b laser products as defined by USHHS, CDRH/FDA, OSHA and EN standards and regulations known to be effective at the date of manufacture.
Every hazardous situation cannot be anticipated, therefore, the user must exercise care, common sense, and observe all appropriate safety precautions applicable to Class 3b lasers and high-voltage electrical equipment during installation, operation and maintenance.
Deviation from published operating or maintenance procedures is not recommended. Operation and maintenance procedure changes are

performed entirely at the user's risk.

SOFTWARE LICENSE
The software described in this document is furnished under a License Agreement, which is included with the product. This agreement specifies the permitted and prohibited uses of the product.

Starting up the system

Starting the Windows NT™ Operating System

You don't have to start Windows NT™–it starts automatically when you turn on your PC. You will first see a splash screen.
Next you have to log on to your computer. As you can see from the instructions in the box, pressing the **Ctrl**, **Alt** and **Delete** keys at the same time will log you on.
After pressing the **Ctrl**, **Alt**, and **Delete** keys, the **Login** information dialog box appears.
Typing your password identifies you as a valid user for this computer.
The default user name for the Leica TCS SP2 system is "TCS_User."
A standard password was not set. It is recommended setting up a separate user ID for each user (system administrator). This will create individual directories that can be viewed only by the respective user.
Since the LCS software is based on the user administration of Windows NT ™, separate files are created for managing user-specific profiles of the LCS software. For information about setting up users under Windows NT ™, please refer to the chapter "Setting Up Users under WinNT" in this manual.
After logging on with your user ID, you may change your password by pressing the keys **Ctrl**, **Alt**, and **Delete** at the same time.
Then click on **Change Password**. The **Change Password** dialog box displays.
Type your current password in the **Old Password** field (passwords are case sensitive, so be sure you use the right case). Then press the Tab key. Pressing the Tab key moves the cursor to the next field.
Type your new password, then press the Tab key again. Confirm your new password by re-entering it. This will eliminate any typing errors. This is especially important since the characters you type appear as asterisks on the screen.

Note

If you miss-type the confirmation password, you will see a warning dialog. Try again!

Then click the OK button. Your new password will be in effect the next time you log on.

Caution

Do not forget your password if you set one! Without the right password you cannot access your computer anymore.

The Welcome dialog box is now displayed. Take a moment to read the "Did you know..." tip and then click the **Close** button to begin using Windows NT.

Using a Mouse

You need a mouse to work most efficiently in Windows NT. Here are the mouse actions you need to know:
Point means to move the mouse pointer onto the specified item by moving the mouse. The tip of the mouse pointer must touch the item.

Click on an item means to move the pointer onto the specified item and press and release the mouse button once. Unless specified otherwise (i.e. right-click), use the left mouse button. Clicking usually selects an item.
Double-click on an item means to move the pointer to the specified item and press and release the left mouse button twice quickly. Double-clicking usually activates an item.
Drag means to move the mouse pointer onto the item, hold down the mouse button, and move the mouse while holding down the button. Unless specified otherwise (i.e. right-drag), use the left mouse button.

The Windows NT Interface

The basic interface of Windows NT is called the "**Desktop**". It provides a background for the items it contains.
The initial icons on the desktop allow the user to view and interact with the system in a logical way.
The Windows NT screen contains many special elements and controls. Here is a brief summary:
The background on which all the pictures and boxes rest, is the desktop.
The taskbar shows the windows and programs that are open. You can switch between open windows and programs by clicking the name on the taskbar.
The Start button opens a menu system from which you can start programs. Click on the Start button, then click on your selection from each menu that appears.
Some icons appear on your desktop. You can activate one by double clicking on it.
We now take a brief tour of the items you see on the screen.
A standard desktop item is the **My Computer** icon. **Double-clicking** this icon opens the My Computer window.
The 'My Computer' window gives you easy access to the major components like hard and floppy disk drives of your computer system or workstation. For example, by double-clicking the **Hard disk [C:]** icon you can see the contents of your PC's hard drive. This allows the user to view local resources as objects. The Windows NT Workstation 4 control panel and print control/support are also accessible from 'My Computer.' If you installed one of the additional applications such as 'Dial-Up Networking' at installation time, it will also appear in the 'My Computer' window.
You can use the **Control Panel** icon in the 'My Computer' window to view and change any system component. The Control Panel contains numerous icons that allow you to control your system. The particular icons that you see on your PC may be slightly different from those illustrated, due to that fact that you may have different hardware installed, and may or may not be connected to a network or modem. You may also have different Windows NT Workstation 4 options installed.
Double-clicking the **Network Neighborhood** icon displays the Network Neighborhood dialog box which gives you information about who and what is connected to your workstation. It provides an easy mechanism for browsing any network systems and resources that you may be able to connect to in a way that is independent of the actual type of network vendor. Traditionally, if a system needed to be simultaneously connected to different types of network, the way in which each could be connected and viewed would be vendor-specific. Windows NT Workstation 4 is capable of displaying a common view of your entire network even though it may actually comprise resources from Windows NT, Novell NetWare, Banyan Vines, or others!
The **Inbox** icon is used if Microsoft Exchange is active on your system. Windows NT Workstation 4 has built-in electronic mail services based on

Microsoft Mail (MS Mail) and Microsoft Exchange. If there is already an MS Mail post office on the same network that the system is connected to, the Windows NT Workstation 4 mail client can connect directly to it. The Inbox lets you access your messages.
The **Recycle Bin** icon represents the holding place for deleted items. As long as files are in the Recycle Bin they can easily be recovered if they have been accidentally deleted. Windows NT Workstation 4 will preserve files until the system runs out of free disk space. When this happens Windows NT Workstation 4 will prune the contents of the Recycle Bin on a first-in first-out basis.

Caution

Files that are overwritten due to an application using a duplicate filename will not be saved to the Recycle Bin.

Double-clicking the Bin displays its contents. The empty window confirms that there are no items in the Recycle Bin.

The Start Menu

A single click of the left mouse button on the Start button opens the start menu and presents the seven major option categories for starting work on the system.
A single click of the right mouse button opens a small but powerful control menu containing the options Open, Explore and Find.
Their functionality is described below.

Starting a Program

The Start menu contains the various categories where your applications and work are stored. You can move further into the various subcategories by positioning the mouse over the category that you are interested in to automatically open the next subcategory. You do not have to click the mouse!
The **Programs** command displays the Programs menu. This menu lists all of the applications installed and available to you. Some options are marked by an arrow. This arrow indicates that a submenu follows. Drag the mouse cursor over the **Accessories** command to see its submenu. The Accessories submenu lists the set of Windows NT built-in programs.
TIP: If you drag an object either from the Desktop or Windows Explorer and drop it directly onto the Start button, a link to that object will automatically appear in the Start menu.
There are many ways to start a program. The following method is the simplest one:
1. Click on the Start button.
2. Click on Programs.
3. Click on the group that contains the program you want to start (for instance, LCS).
4. Click on the program you want to start (for instance, Leica LCS).

Another way to start a program is to open a document that you created in that program. The program automatically opens when the document opens. Double-click on a document file in My Computer or Windows Explorer to open it. Or click on the Start button and select a recently used document from the Documents menu.
You can also start a program by double-clicking on its shortcut icon on the desktop. Shortcut icons are links to other files. When you use a shortcut, Windows simply follows the link back to the original file. Whenever you use a document or program frequently, you might consider creating a shortcut for it on the desktop. To do so, just use the right mouse button to drag an object out of Windows Explorer or My

Computer. After releasing the mouse button, a menu appears. Select the option Create Shortcut(s) Here. Some programs automatically create a shortcut during their installation procedure.

Caution

Windows NT Workstation 4 does not actively track a link between an original and a shortcut. For instance, if you create a shortcut of a program, and subsequently move (rather than copy) the original to a different folder, then the shortcut may no longer function.

The Startup folder is special in one respect: any programs located in this folder will start automatically when Windows NT Workstation 4 is started. The Documents menu shows the names of the 15 files you created most recently. You can open any of these files and its related application at the same time by clicking the file's name in this menu.

Caution

Document files that are opened within an application (typically by selecting the File/Open command within the application) will not be displayed here. Only documents opened directly from the Desktop will be displayed here.

The Settings menu offers three commands for changing your system's settings. You can directly access the Control Panel and Printing folders. Also accessible is the Task Properties window.
Being able to access the core system configuration utilities in this way is particularly useful when an application is already in the foreground and you want to make a quick change.
The Find command features an easy way to locate all system resources. Within the Find category you can perform searches for three distinct types of search as described below:

The Taskbar

The taskbar–positioned at the bottom of the screen–provides a constant view of which applications are running on the system and an easy way of switching between them. As you add to the number of concurrently running applications, the taskbar automatically re-sizes its iconized view of the applications to ensure that they can always be seen. To switch from one running program to another, simply click on the second program as displayed in the taskbar.
The taskbar also provides constant additional information such as the system time and volume control if a sound card is installed. All of these functions can be adjusted by the user.

Setting the Time and Date

The current Date, Time and Time Zone information can be set from the Date/Time icon within the Control Panel. This setting is important since Windows NT stamps the date and time on all of your files as you create and modify them. The two options can be selected by clicking on the appropriate tab.

To change the Date and Time

Click on the appropriate date or use the controls to change the month or year. The time can also be changed by first selecting the digital display and then using the up and down arrows.

To change the Time Zone

Select the appropriate Time Zone from the drop down list at the top of the

screen. Notice that the option to automatically adjust the clock for daylight savings time is selected. On some systems you can also drag the highlighted area on the world map and drop it on the correct location. Changing the date and time information within Windows NT Workstation 4 will update the battery backup CMOS clock in your system.

Note

Depending on the shell configuration, systems connected to a network may get a time and date update from a network server every time they log on. If the server's time is incorrect, your workstation's time will be wrong, too. Please inform your network administrator.

Getting Help

Windows NT includes a powerful help system. In addition to Help menus in every window, there is a standalone Help feature available from the Start menu. To access it, click your mouse on the Start button, and click on Help.
There are three tabs in this box: Contents, Index, and Find. The Contents tab appears on top. To move to a tab, click on it.

Contents

The Contents tab displays individual Help topics. The topics are organized into categories and are represented by small book icons. Double-click on any book to open it. Sub-books and documents appear. Double-click on sub-books and documents to open them.

Index

The Index tab displays an index of all available topics. Type the word you want to look up. The Index list scrolls to that part of the alphabetical listing. When you see the topic on the list that you want to read, double-click on it.

Find

Rather than searching for information by category, the Find tab offers a full text search. Enter the word(s) or phrase to be searched for under Help in the text entry box. The text entry box is linked to a list of words in your Help files, and any words or phrases that match will be displayed. You can specify more than one word by separating words with a space. If you wish to change a search option, select Options. The first time you click on this tab, Windows tells you it needs to create a list. Click Next and then Finish to allow this. The main Find tab appears next. Type the word(s) you want to find in the top text box. Then click a word in the middle box to narrow the search. Finally, review the list of help topics at the bottom, and double-click the one you want to read.
When you're done reading about a document, click Help Topics to return to the main Help screen, or click Back to return to the previous Help topic. Click the window's Close button to exit Help.

Shut Down Windows NT

Always use the **Shut Down** command before you turn off your PC. The Shut Down option allows the user to close the Windows NT Workstation operating system and ensure all running processes can halt cleanly and are given the chance to flush any data out to the disk that may be in cache memory. Several options are available when shutting the system down.

Caution

Powering down your computer without prior shutting it down may result in severe data loss.

Setting Up Users Under WinNT

1. Logging on as administrator
Log on as administrator by using the ID "Administrator" and the password "Admin."
2. Opening the User Manager
Select ***Start → Programs → Administrative Tools → User Manager*.**
3. Defining a new user
Enter at least the following information in the opened dialog window:
1. User name
2. Password (must be re-entered in the following line for confirmation purposes)
Select the following two check boxes:
a.) "User must change password at next logon" (this allows the new user to define his or her own password at logon)
b.) "Password never expires" (this allows a defined password to be valid either until it is changed in the User Manager or the user is deleted)
Select the "Profiles" option in the bottom section of the dialog. In the "Local path" field enter the following path for storing user-specific files: d:\users\username ("username" is an open parameter which must be replaced by the currently defined user name).

Note

Factory installed hard disks feature 2 partitions (C:\ and D:\).The user directory should be set up on partition D:\.

The Leica Confocal Software: Overview

Starting the Software

Requirements for Starting the Software

The LCS software is copy-protected to prevent it from being used on two computers at the same time. This protection system allows all additional application packages to be used. The protection system consists of a dongle that must be inserted into the parallel port of the workstation. The functionality of the parallel port (e.g., for printing, etc.) is not affected. To use the software on a second computer, the dongle must be attached to its parallel port.

Note

If you remove the dongle from the workstation of the confocal system, the software cannot be started, thereby preventing operation of the confocal system.

The LCS software can be started in two operating modes: hardware mode and simulation mode. In hardware mode, all hardware components are accessed and initialized by the software. For this reason, you should switch on the hardware first and then wait about 20 seconds before starting the software.
In simulation mode, the software runs independently of the hardware. This mode is intended for secondary installations on another computer, for example for training or offline analysis of existing data.

Starting the Software

Select **Start|Programs|Leica Confocal Software**. The initial screen of the Leica Confocal Software appears. This window allows you to select from three profiles.

Company
This option starts the Leica Confocal Software using the default factory settings. This means that the configuration and the position of the Toolbars is preset. These settings cannot be changed.

Personal
This option lets you use a user-defined configuration profile. The user name is dependent upon the account used to log on to the operating system. If the custom profile does not yet exist at the first initialization, the default factory settings are applied to the personal profile.

Last Exit
This option loads the configuration profile that was last active.

For advanced users:
If you have more than one configuration profile, you can load them at startup by clicking the button with the three black dots, which is located at the right lower edge of the profile options. You may also use this option to reset your custom configuration profile to the standard factory profile.
Clicking the Start button starts the Leica Confocal Software using the corresponding configuration profile.

Note

After a delay, the software starts automatically and loads the currently selected configuration profile.

The Experiment Software Concept

The Leica Confocal Software allows you to join image data or edited images into groups. Each group is called an "experiment" and is stored in a special data format (*.lei). This makes it possible to store both the original experimental image data and the image viewing data together. Additional information can be found in the chapter "Organizing Data by Grouping Experiments"

The Basic Structure of the User Interface

The appearance of the **G**raphical **U**ser **I**nterface–henceforth referred to as GUI–depends strongly on the configuration profile being used. A number of elements of the GUI, however, are standard.
The GUI provides the following standard elements:

The menu:
The menu contains the submenus File, View, Macro, Tools, Window and Help. The submenus contain commands and information for general display, settings and user customization. They do not provide any of the functions for directly controlling the scan functions. These functions are located in the TCS menu (***View → Menu → TCS Menu***). The menu line cannot be customized.

The Viewer window (TCS_Viewer):
The Viewer window displays the image data, experimental conditions and user data. The image window can be configured (see the chapter on "Modifying the User Interface and Defining User-Specific Settings"). The image window shows not only the confocal image data records, but also the experiment data and system settings. You can open a new experiment image window by selecting ***File → New***.

The TCS menu (TCS_Menu):
The TCS menu contains buttons for the individual device functions. It is subdivided into individual work steps. The number of work steps available depends on the installed software. The standard set of work steps consists of data recording (Acquire), image viewing (View), surface reconstruction (3 D), measurement functions (Quantify), image processing and analysis functions (Process), and documentation functions (Annotate). If the TCS menu is not displayed using the current configuration profile, you can activate it by selecting ***View → Menu → TCS Menu***.

The toolbars:
This area allows you to insert and customize individual (function) buttons. The advantage of the container is that it can be switched on or off with its entire contents of buttons. To do so, select ***View → Menu → Container***.

The document viewer window (Experiment Overview):
The document viewer window displays the recorded experiments and their contents in a directory tree. Open this viewer window by selecting ***View → Experiment Overview***.

The status bar:
The status bar is found at the bottom edge of the Leica Confocal Software user interface. It displays:

- the progress while loading image data (progress bar)
- the version number of the software
- the name of the system configuration (system type)

For details about these functions, refer to the chapter on "Software Reference".

Opening Data Records

Readable File Formats
The following file formats can be opened and viewed in the Leica Confocal Software:
Experiment (*.lei):
The format of this type of file is Leica-specific and binary. This format is provided for the data of entire experiments.
Tiff files (*.tif):
These are Leica image files in single and multi TIFF format. Both image files in the previously used TCS formats and external files in RGB TIF format can be read.
Annotation (*.ano):
The format of this type of file is also Leica-specific and binary. It is used for saving annotation sheets. The elements on the annotation sheet, such as images, texts and graphic images, are each available as individual objects.
When these files are loaded, both the image data and the experiment settings are loaded.
Automatically Applying the Study Parameters of an Experiment
With the Leica Confocal Software, hardware settings that have been saved for previous experiments or single images can be applied to new experiments. This allows you to carry out different experiments using constant settings. To carry over settings from a previous experiment, open the viewer window for the data record that contains the settings you want to apply. There, click the "Apply" button, which, in the factory default configuration profile, is located in the toolbar.

Note

*If you do not find the "Apply" button in one of the open windows, you can load it into any of the windows by selecting **Tools** → **Customize**. In the dialog window that appears, select the "Commands" tab under the File category. With the left mouse button, click the "Apply" button and drag it while holding the mouse button pressed to the desired window. Release the left mouse button to insert the button at the current location.*

Saving Images

As described above under "Readable File Formats", individual images and experiments can be saved using the same data formats.
Select ***File*** → ***Save*** to save your images and experiments. The first time an experiment is saved, the "Save as" dialog opens automatically, allowing you to specify a file name. In addition to defining a suitable file name, you can also select a file format here. Experiments can only be saved in the Leica-specific *.lei format. When you are saving experiments, you may be able to save existing individual images in *.tif or *.raw format.

Note

If you are saving an experiment or image that has already been saved before, the old data will be overwritten.If you do not want to do this, select **File** → **Save as** *to save the new or changed data to a new file with a new name.*

Data Organization by Grouping Experiments

The concept of the Leica Confocal Software allows you to group individual images, image series and the results of edited images into single groups, or experiments. The Experiment Overview window provides a general overview of the open experiment. To open the Experiment Overview window, select ***View → Experiment Overview***. Create a new experiment by selecting ***File → New*** or ***File → New (Template)***. Files that were previously saved are handled as separate experiments when opened.

Compiling Experiments

After you have defined a new experiment by selecting ***File → New*** or ***File → New (Template)***, you can start filling it with data.

Note

Images that have been recorded in continuous scan mode are overwritten automatically when the next scan is started. If you want to keep a single recording as a permanent part of an experiment, you should use the single scan function.

An experiment contains data acquired with the "single scan" or "series scan" function. If you carry out image processing functions on a data record, you can save the results as additional components of the experiment. To do this, double-click the desired image or series in the experiment overview window. Carry out the desired image processing functions (such as maximum projection or topological image or others). Mark the region within the viewer window that you want to keep as part of the experiment. Click the right mouse button (to open the context menu) and select ***Send to → Experiment***. The ***Selection (raw)*** option stores a copy of the raw data of the selected object as a new, separate component of the experiment. The ***Selection (snapshot)*** option stores an RGB image (no 3D data, just a pure snapshot) of the selected object as a new, separate component of the experiment.

Menu Functions

The following TCS SP2 functions can be carried out from the menu bar:
File Menu
New:
This option opens a new experiment in a new window. The display properties of the new window are determined by the active defaults (see the chapter on "Default Settings and Templates").
New (Template):
This option opens a new experiment in a new window. The way in which the viewer window is displayed can be selected from a set of templates.
Open:
This option opens a previously saved experiment or a single data block (image data or annotation). The display properties of the new window are determined by the active defaults (see the chapter on "Default Settings and Templates").
Open (Template):
This option opens a previously saved experiment, a single data block or annotation files. The way in which the viewer window is displayed can be selected from a set of templates.
Close:

This option closes the current experiment. It does not save the experiment automatically before closing it.

Note

You are not asked whether you want to save the experiment before it is closed.

Close all:
This option closes all open experiments. It does not save the experiment automatically before closing it.

Note

You are not asked whether you want to save the experiment before it is closed.

Save:
Saves the current experiment.
Recent files:
Opens one of the recently opened files.
Print:
Opens a dialog for printing the content of the currently active window.
Exit:
Terminates the program.

The View Menu
Menus:
Use this option to switch TCS menu and the toolbar on and off.
Status bar:
This option switches the display of the status bar on and off at the bottom edge of the user interface of the LCS software.
Experiment Overview:
This option opens a window displaying the individual experiments and their components (data records, graphs, etc.) in the form of a tree. Double-click an experiment component within the Experiment Overview window to bring the corresponding viewer window to the foreground.
Viewer Options:
Use this item to configure various options for displaying data records in the viewer window, such as the display angle and calculation methods for displaying image data, etc.

The Macro Menu
Macros:
Depending on the software installed, either use this option to edit macros directly or just list and run them.

Note

You can edit and modify macros only if the complete, integrated VBA development environment (IDE) is installed.

Record a New Macro:
This starts the automatic macro recorder. For more information, refer to the documentation provided with the optional macro development environment.
Pause Recording:
This pauses the macro recorder.
Stop Recording:
This stops the macro recorder.
Visual Basic Editor (optional):
This option launches the software for developing macros and programs

based on Visual Basic for Applications (VBA). Here you can edit and modify macros and even develop entire programs.

The Tools Menu
Legend Info:
This option opens the dialog window for entering user-specific data. This data (e.g., name of institute, name of specimen, etc.) is saved with each data record and can be displayed next to the acquired images in the legend.
Objective:
This option opens the dialog window for defining applied objectives. The objectives you use can be made known to the software by copying them using "drag and drop" from the list provided in this dialog window.

Note

It is important to apply the correct data for the objectives, as some of the calculated sizes (such as the scan field dimensions) depend on them.

Microscope:
Use this option to select the Leica microscope you are using. This setting influences the way in which the microscope is controlled (for example, the motorized objective nosepiece).
Settings:
Use this option to select the hardware settings that you want to be applied when opening a previously saved data record.
License:
This option allows you to install subsequently purchased licenses. A subsequent license usually is installed if a software package offered as an option is purchased.

The Window Menu
New Window:
This option opens a new viewer window within the same experiment. For example, you can use this function to view the same data record using different views (such as topographical and overlay views).

The Help Menu
Contents:
Opens the table of contents of the online help.
Search:
Starts the search function for the online help.
Index:
Opens the index of the online help.

Keyboard Shortcuts

Special key combinations have been defined to speed up access to frequently used software functions.

Keyboard Shortcuts	Function
F1	Opens the online help.
ALT + F8	Opens the Macros dialog window for running, editing and deleting macros.
ALT + F11 (optional)	Starts the VBA development environment (optional).
CTRL + L	Opens the Legend Info dialog window for entering user-specific settings that can be applied when documenting, saving and viewing the documentation of image recordings.
CTRL + J	Opens the Objective dialog window for defining and selecting applied microscope objective lenses.
CTRL + N	Opens a new experiment.
CTRL + O	Starts the Open dialog window for opening an existing file.
CTRL + P	Opens the Printer Selection dialog window.
CTRL + S	Saves the active experiment.

LCS Software Functions

Introduction to the Leica Confocal Software Help

Three different help levels are available for the Leica TCS SP2:

Quick Help
When you let the mouse pointer hover over a Leica Confocal Software button, a brief explanation of the function of this button is displayed. This so-called Help Banner automatically disappears when the mouse pointer is moved.

Context-sensitive Help
If you want to use the context-sensitive help function, click on the Help button:

The entire user interface is then frozen and a question mark appears beside the mouse pointer. Then, instead of triggering the button function, clicking a button opens an explanation of the button's function. If the Help button is not present on the user interface:

- Select the Customize option from the Tools menu. Here you will find all buttons arranged by categories.
- The Help button can be found in the File category.
- Click on it using the left mouse button and drag it to the desired window.

Contents of the Online Help
Select the Contents option from the Help menu to view the online help table of contents, which allows you to select any function in order to view information on it.

Keyword Search (Index)
Select the Index option from the Help menu to view an index of key words. Select a key word. View the corresponding content pages by double-clicking the key word or selecting it and then clicking the Display button.

Full-text Boolean Search
Select the Search option from the Help menu to launch the full-text search engine. Enter your search word in the input field. Click the triangle to the right of the input field to view the available logical operators. Select the desired operator. Enter the second search word you would like to associate with the first search word behind the operator:

Examples	Result
Pinhole AND Sections	This phrase finds help topics containing both the word "pinhole" and the word "sections".
Pinhole OR Sections	This phrase finds help topics containing either the word "pinhole" or the word "sections" or both.
Pinhole NEAR Sections	This phrase finds help topics containing the word "pinhole" and the word "sections" if they are located within a specific search radius. This method also looks for words that are similar in spelling to the words specified in the phrase.
Pinhole NOT Sections	This phrase finds help topics containing the word "pinhole" and not containing the word "sections".

Favorites
If you select Favorites in the dialog window of the online help tab, you can include the current help topic in a list, making them easily available for future use.

Opening the Context-Sensitive Help

Function
Click Help to open the context-sensitive online help function, which provides you with short explanations for the various buttons and functions of the Leica Confocal Software.

- Click the Help button.
- A question mark appears next to the mouse pointer. This temporarily disables the functions of all buttons.
- Use the mouse pointer to click the button that you want an explanation of.
- Online help opens directly to the description for the corresponding button.

Online help also provides you with an index of key words and a search function so that you can search for specific topics and buttons. Furthermore, you can print the individual descriptions.

You can also open the online help by selecting Contents, Search or Index option under the Help menu.

Documentation Conventions

Button
The buttons provided on the user interface of the Leica Confocal Software. Buttons are marked with icons and/or have an (often abbreviated) English label. They either trigger actions directly or open dialog windows.

Menu
The menus are divided into the categories of File, View, Macro, Tools, Window and Help and are displayed in the menu bar located at the upper edge of the user interface.

Option
Options refer to the selectable items that are hierarchically listed below the menus. Options either trigger actions directly or open dialog windows.

Dialog Window
Both buttons and options open dialog windows. Dialog windows are used to set various parameters and select functions.

Register
Registers are found in dialog windows. Registers thematically combine the parameters and functions that can be configured in dialog windows. Some registers are divided into fields.

Viewer window
The Leica Confocal Software contains two types of viewer window. The Viewer window is called up by pressing the New button and displays the recorded images. The Experiment Overview viewer window displays the recorded images in a directory tree. This viewer window is called up from the View menu and appears as a separate window at the left side of the user interface.

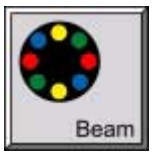
Legend
The Leica Confocal Software provides two legends, which display the parameters and settings of an image recording. The Experiment legend can be placed at the right edge of the Viewer window. The Hardware legend is called up from the View menu and appears as a separate window at the left side of the user interface.

Context Menu
Context menus appear when you click the right mouse button while holding the mouse pointer over certain areas of the user interface. Context menus contain various, context-sensitive commands.

see ...
This symbol is a reference to another topic in the online help.

Data Recording

Setting the Beam Path



Function
Open the Beam Path Setting dialog window using the Beam button, and set up the beam path and the detectors with the corresponding color look-up tables used to record the image.

Selecting the excitation wavelength
Above the spectrum in the dialog window you will find boxes to set the excitation wavelength using an AOTF:

- Click the check box next to the laser name to activate the laser.
- Set the output for the laser line by moving the slide on the corresponding scale to the desired value, or
- Double click the percentage value for the laser output. This opens a second dialog window where you can enter an exact value.
- The active laser line is shown as a line in the spectrum.

Loading and Saving Parameter Settings
Also above the spectrum is a list box for loading and saving parameter settings. The manufacturer has predefined parameter settings for the most important fluorescent dyes. They are labeled with the letter **L** (for Leica) and can be loaded only, not modified.

You can also save the settings made for a specific image recording as user-defined parameter configuration so that you can load them any time with a single click:

- Click the Save button.
- This opens a dialog window for entering a name for the parameter configuration.
- The parameter configuration is stored in the list box under User and is labeled with the letter **U** (for User).

Furthermore, you can individually select the parameters that you want saved when using the Save command:

- Select the Settings option from the Tools menu.
- In the Settings dialog window, click the Instrument Parameters tab.
- Select individual parameters by clicking the corresponding checkboxes. The Select all and Deselect all buttons allow you to select all or no parameters quickly.

Click one of the user-defined or preset parameter configurations and then press the right mouse button to open a context menu, which contains the following commands:

Command	Function
Set as default setting	The standard setting for the parameters is loaded when you start the software.
Remove default setting	This removes the default setting property from the selected configuration.
Load	Loads the parameter configuration.
Rename (only for **U**)	Use this to change the name of the parameter configuration.
Delete (only for **U**)	Deletes the parameter configuration.

Selecting the excitation beam splitter
The symbol (green, tilted line) for the excitation beam splitter is located to the left of the spectrum. Click on the symbol and select the desired beam splitter:

- Neutral filters, such as the RT 30/70 filter, are used in reflection applications to lead the excitation light to the sample and the reflected light to the detection pinhole.
- Dichroic filters, such as the reflection short pass filter RSP 510, are used in fluorescence applications to split and detect fluorescent light of a particular wavelength range from the excitation light.
- Double dichroic filters are used for image recording, where the preparation is marked with two fluorescent dyes and two excitation wavelengths. The DD 488/568 double dichroic, for instance, is used to split excitation light with a 488 nm and 568 nm wavelength from the detection light. Depending on the system configuration, you can also select DD 488/ 543 and DD 458/ 514 type double dichroites.
- Triple dichroic filters are used for image recording, if the preparation is marked with three fluorescent dyes and three excitation wavelengths. The TD 488/568/633 triple dichroic, for instance, is used to split excitation light with a 488nm, 568nm, and 633nm wavelength from the detection light. Depending on the system configuration, you can also select TD 488/ 568/ 647 and TD 488/ 543/ 633 type triple dichroites.

Setting detectors and color look-up tables (LUT)
Below the spectrum in the dialog window the boxes for the four detectors, PMT 1 to 4, as well as for the PMT Trans transmitted light detector are shown. Adjust the settings as follows:

- Activate each of the desired detectors by clicking on its corresponding Active check box below the color look-up table icon. A cast shadow now links the activated detector to the corresponding slide bar on the scale of the spectrum.
- Specify the wavelength range for the detection by dragging the two ends of the slider to the desired positions on the scale, or
- Double-click the slide bar to open an additional dialog window in which you can enter a precise value for the start point and the end point of the wavelength range.
- For fluorescence applications, list fields with common fluorescence dyes have been set up for each detector. Select the desired fluorescence dye to display its emission curve in the spectrum.
- Select a color look-up table by clicking on the corresponding icon.

see Selecting Color Look-Up Tables

Selecting an Objective

Function
Click the Objective button to open a list of objectives and make an appropriate selection. This list contains only the objectives that were previously assigned to one of the maximum of seven threaded ports on the objective nosepiece. You can assign objectives as follows:

- Select the Objectives option from the Tools menu. A dialog window opens containing an extensive list of objectives and the symbolic representation of the slots on the objective nosepiece.
- Find in the list the objective that you are using and select it with the mouse. Click and hold the left mouse button and drag the objective onto the symbol representing the slot in which the objective is installed.
- The assignment is saved in the software and the objective appears in the selection list, which you can open by pressing the Objective button.
- Repeat this procedure for every objective that you have installed in the objective revolver.

You can use the Add, Remove and Edit buttons in this dialog window to add new objectives, remove objectives or edit existing objective labels.

Note

The microscope models DM RXA, DM RXE, DM IRBE, and DM IRBE2 use a software program to control the objective nosepiece. The program automatically rotates the selected objective into the beam path whenever you select an objective by using the button or Objective dialog window. All other microscope models require not only software adjustment of the objective, but also manual rotation of the objective into the beam path.

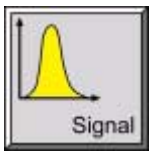
Additional information
When selecting the correct objective for a specific application, the objective's correction class (achromatic, apochromatic, fluorite objectives and plane objectives) and especially the magnification factor and the numerical aperture are of great significance. The numerical aperture determines the resolution capacity of an objective and is deduced from the flare angle of the light cone recorded by the objective and the refraction index of the medium between objective and specimen: NA = n*sin a

Objectives with greater magnification generally have larger numerical apertures but smaller entrance pupils and therefore can record light only from a relatively small scan field. Objectives with larger apertures permit higher resolutions but allow less free working distance. The following table illustrates this relationship:

Objective	Resolution (xy)	Resolution Air (z)	Resolution Water (z)	Resolution Oil (z)	Scan Field Size (xy)
HC PL FLUOTAR 5x 0.15	1301	19410	25879	29559	3000
HC PL FLUOTAR 10x 0.30	651	4768	6407	7335	1500
N PLAN 20x 0.40	488	2630	3566	4093	750
N PLAN 50x 0.75	260	649	948	1108	300
PL APO 100x 1.40	139	319	209	236	150
	Values in nm at wavelength λ=488 nm	Values in mm			

Typical Applications
Material scientists commonly use dry objectives to study surface structures. Immersion Objectives are the best choice for producing images of layered structures in which material layers with varying refraction indexes come into contact with each other. When working with biological specimens, the choice between an oil immersion objective and water immersion objective depends on the specimen itself and its embedding medium. You will achieve the best resolution when the refraction indexes of the embedding medium and/or the specimen are matched to the refraction index of the objective medium.

Setting the Detectors



Function
Use the Signal button to open a dialog window for adjusting the detectors so that the entire range of intensities of a color look-up table is assigned and displayed in the image. For this purpose, a Gain value and an Offset value can be set for each detector. The gain value modifies the amplification of the detected signal, thus changing the brightness and contrast of the image. The offset value defines a threshold value. Only those signals that lie above the threshold value are detected and displayed in the image.

There are two methods for setting the gain and offset values:

- Use the mouse to move the slide on the scale. The corresponding value is shown below the corresponding scale.
- Double-click the numerical value that is displayed below the scale. This opens a second dialog window where you can enter an exact value.

Best suited to optimization of gain value and offset values are the color look-up tables *Glow Over*, *Glow Under* and *Glow Over and Under*.

see Selecting Color Look-Up Tables

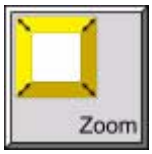
You can also set the gain value and offset value of the detectors using the corresponding radio buttons of the control panel.

see Controlling Functions Using the Control Panel

Note

A detector is not enabled unless its corresponding Active checkbox is checked in the Signal or Beam Path Setting dialog windows.

The Electronic Zoom



Function
In confocal microscopy, the magnification of an image is determined both by the objective and the electronic zoom. The objective generates a first image whose magnification is based on the objective's magnification factor. The electronic zoom provides additional magnification. A zoom factor of 1 scans the maximum scan field size with a specific number of dots. If the zoom factor is set to 2, the same number of dots is used to scan a scan field with half the page length of the maximum scan field (1/4 the original scan field). This achieves stronger magnification and thus better image resolution too, because a smaller scan field is scanned with the same frequency, which results in a higher density of data.

In the dialog window that was opened by pressing the Zoom button, you can select one of the preset zoom factors. If you click on the Others button, you can set a different zoom factor in two ways:

- Use the mouse to move the slide of the scale. The corresponding value is shown in the middle of the dialog window.
- Double-click the boldface numerical value located in the middle of the dialog window. This opens a second dialog window where you can enter an exact value.

You can also set the zoom factor using the corresponding rotary knob on the control panel.

see Controlling Functions via Control Panel

Additional information
While zoom factors from 1 to 32 can be set, the useful magnification of the electronic zoom cannot be increased infinitely. The limit is achieved using the smallest optically resolvable distance, which is determined by the resolution capacity of the objective. According to the Nyquist theorem, this smallest optically resolvable distance can only be mapped without information loss if it is scanned with about 2 to 3 raster points. If the scan frequency is exceeded at a relatively high zoom factor and the specified scan format, further magnification is no longer useful because no more optical details can be resolved (zero-order magnification).

see Selecting a Scan Format

see Selecting an Objective

Note

With bleach-sensitive specimens it is advisable to restrict the use of the electronic zoom to short time intervals. Since you are recording a segment of the specimen with a relatively higher scan frequency in the case of high zoom factors, the specimen is exposed to a stronger impact of light. This can lead to photomechanical destruction of the specimen (optical bleaching).

Optimal Zoom Factors

The following table shows which zoom factors (marked red) - depending on the scan field size and scan format specified by the objective that can be used to scan the specimen without loss of data:

Objective			Scan Format	Zoom Factors	Scan Field Size (mm)	Scan Frequency (nm)	Resolution/ Scan Frequency
Magnification	Numerical Aperture	Resolution at λ=488 nm					
20	0,6	325	1024 x 1024	1	750	732	0,4
				2	375	366	0,8
				4	187	183	1,8
				6	125	122	**2,7**
			512 x 512	1	750	1465	0,2
				2	375	732	0,4
				4	187	365	0,9
				8	94	183	1,8
				10	75	146	**2,2**
			200 x 200	1	750	3750	0,1
				2	375	1875	0,2
				4	187	935	0,3
				8	94	470	0,7
				16	47	235	1,4
				32	23	115	**2,8**
40	1,25	156	1024 x 1024	1	375	366	0,4
				2	187	183	0,8
				4	94	92	1,7
				6	62	60	**2,6**
			512 x 512	1	375	732	0,2
				2	187	365	0,4
				4	94	183	0,8
				8	47	92	1,7
				10	37	72	**2,2**
			200 x 200	1	375	1875	0,1
				2	187	935	0,2
				4	94	470	0,3
				8	47	235	0,7
				16	23	115	1,3
				32	12	60	**2,6**
100	1,4	139	1024 x 1024	1	150	146	0,9
				2	75	73	1,9
				3	50	49	**2,8**
			512 x 512	1	150	293	0,5
				2	75	146	0,9
				4	37	72	1,9
				6	25	49	**2,8**
			200 x 200	1	150	750	0,2
				2	75	375	0,4
				4	37	185	0,7
				8	18	90	1,5
				12	12	60	**2,3**

Note

The Leica Confocal Software provides three different zoom functions: the electronic zoom, the 3D zoom and the graphic zoom.

see Zooming the 3D View
see Viewer Options Dialog Window, Display Icon

Enlarged Recording of a Frame

Function
The Zoom In function enables an enlarged recording of a freely selectable square frame of a specimen.

- Click the Zoom In button. At the same time, the Rectangle button is automatically activated.
  see Defining the Region of Interest (ROI) as a Rectangle
- Specify the frame to be enlarged by drawing a rectangular evaluation area (region of interest or ROI) into the image using the Rectangle button.
  see Selecting and Moving the Region of Interest (ROI)
- Use the Select button to move the frame on the image.
- To start the image recording, press the Continuous Scan, Series Scan or Single Scan button.

Note

The software automatically adapts the freely selected rectangular frame to the actual square form that can be implemented by the scanner. The dashed line shows the actual frame.

Additional information
The enlarging recording of a frame will only provide additional detailed information as long as the distance of the grid points remains larger than half the optical resolution. On the other hand, the resolution depends on the objective used, the adjusted scanning format, and the light wavelength used (Empty magnification).

Typical Applications
All applications that deal with the optical resolution of very small structures and that can do without an overview image. While recording such specimens, care should be taken that an objective with a corresponding high optical resolution is used for image recording.

Setting the Detection Pinhole

Function
Clicking the Pinhole button opens a dialog window that you can use to set the diameter of the detection pinhole. In the upper right list field in the dialog window, select the units for displaying the diameter. You can select from mm, Airy units and digital values. There are two ways of specifying a value for the diameter:

- Use the mouse to move the slide on the scale that is displayed on the left in the dialog window. The corresponding value is shown in the middle of the dialog window.
- Double-click the boldface numerical value located in the middle of the dialog window. This opens a second dialog window where you can enter an exact value.

The diameter of the detection pinhole must be set to the currently used objective at all times. If you click the Airy 1 button, the detection pinhole is set automatically to the optimum value of 1 Airy unit depending on the objective in use.
In addition to the numerical aperture of the objective and the wavelength of the light, the detection pinhole also determines the thickness of the optical sections.

Additional information
The diameter of the pinhole has the optimum if it matches the diameter of the Airy disc. The Airy disc refers to the inner, light circle of the diffraction pattern of a point light source. The diameter of the Airy disc, in turn, is also dependent on several optical parameters and can be described for the Leica Confocal System as follows:

$$d_{Airy} = \frac{1{,}22 \cdot \lambda}{NA} \cdot M \cdot 3{,}5$$

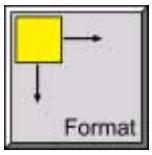
To calculate the diameter of the Airy disc, you need the excitation wavelength l (in the case of several wavelengths, a mean value should be used); the numerical aperture NA and the magnifying factor M of the objective. The factor of 3.6 refers to the magnification of other optical components belonging to the Leica Confocal microscope.

If the pinhole is set to the Airy disc, light from outside the focal plane is suppressed and the signal-to-noise ratio is high. These conditions allow the recording of optical sections of minimum thickness. The wider the diameter of the pinhole, the more light reaches the detector. The image becomes brighter but blurring from structures outside the focal plane will also appear in the image, making it increasingly unfocused.

Increasing the diameter of the pinhole above 1 Airy unit is recommended only for detecting very weak signals.

Generally when recording material scientific specimens, enough light is reflected into the detectors so that the detection pinhole can be closed completely (i.e. set to the minimum diameter).

Selecting a Scan Format



Function
Clicking the Format button opens a dialog window containing a selection list of scan formats. Selecting the scan format selects the image raster that will be used for recording the images. An image raster is the amount of points scanning the specimen in the three directions in space. Besides the numerical aperture of the objective and the excitation wavelength, the scan format, together with the electronic zoom, determines in large part the spatial resolution of the recorded data.

Additional information
When selecting the scan format, observe the influences between the image raster and the resolution of the generated image. According to the Nyquist theorem (or sampling theorem), a structure can only be scanned without information loss if the smallest optically resolvable distance is scanned with about 2 to 3 raster points. This optically resolvable distance, called **lateral resolution**, depends on the numerical aperture of the objective and the wavelength of the applied excitation light:

$$\text{Laterale Auflösung} = \frac{0{,}4 \cdot \lambda}{NA}$$

The following example should illustrate this relationship: You have selected, for example, the PL APO 100x objective unit with a numerical aperture of NA = 1.4 and a wavelength of l = 488 nm. Based on these factors, the smallest optically resolvable distance is determined as follows:

$$\frac{0{,}4 \cdot 488}{1{,}4} = \text{ca. } 140 \text{ nm}$$

Based on this rough formula, the distance of the raster points required avoiding information loss while recording is:

$$\frac{140 \text{ nm}}{3} = \text{ca. } 47 \text{ nm}$$

If however you select a scan format of 1024x1024, a PL APO 100x objective, which has a maximum
Scan field size of 150 mm, would result in the following raster point distance:

$$\frac{150\ \mu\text{m}}{1024} = \text{ca. } 146 \text{ nm}$$

To achieve the required 47 nm raster distance, you can either raise the scan format (to 2048x2048 for instance) or you can decrease the scan field size using the Electronic Zoom (Zoom=2).

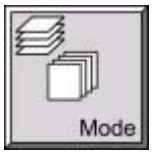
The current raster distance is displayed in the Hardware legend. This value is referred to as Voxel Size and recalculated as soon change the scan format, the Electronic Zoom or the objective.

see The Electronic Zoom
see Selecting an Objective

Note

When the sampling rate of 2 to 3 points is exceeded, it is referred to as oversampling. In the case of oversampling, the structure is fully mapped but no additional information is gained. The disadvantage of oversampling is the long scanning time and the danger of destroying bleach-sensitive specimens. When the sampling rate is less than 2 to 3 points, it is referred to as undersampling. In this case, there is the danger that not all necessary information is sampled. The image might illustrate structures that are not actually present in the specimen. This effect is called aliasing.

Selecting a Scan Mode



Function
Clicking the Mode button opens a dialog window containing a list of available scan modes. The scan mode determines which optical levels of the specimen are to be scanned. Fundamentally, horizontal xy-sections or vertical xz-sections can be recorded. To generate a three-dimensional image of the specimen, the optical sections are continued in the corresponding third direction in space, thus recording a stack of individual images. In addition to this, it is possible to add the factor of time or wavelength while recording the images:

Mode	Function
xyz	An image stack is recorded from the xy-sections in the z direction.
xzy	An image stack is recorded from the xz-sections in the y direction.
xt	A line is recorded several times in succession.
xyt	A xy-section is recorded several times in succession.
xzt	An xz-section is recorded several times in succession.
xyzt	An image stack is recorded several times in succession from xy-sections in z-direction.
xyλ	An xy section is recorded at different wavelengths.
xzλ	An xz section is recorded at different wavelengths.

All scan modes (with the exception of xt) are composed of at least three dimensions. The device will ignore the third as well as other dimensions if you record images only from one optical plane (xy or xz) using the Continuous Scan function of the Single Scan function.

Note

*The xzy and xzt scan modes are only displayed in the list if you previously selected the selection point Galvo using the Z-Scan button. Likewise, the scan modes for a xyλ and xzλ wavelength will only be displayed if only **one** detector is activated in the Beam Path Setting dialog window.*

see Setting the Beam Path

Selecting a Scan Speed

Function
Clicking the Speed button opens a dialog window that you can use to select from four scan speeds.

Speed

200	Image lines per second
400	Image lines per second
800	Image lines per second
1000	Image lines per second

The data recording speed is further increased in combination with the bi-directional scan.

see Unidirectional or Bi-directional Scan

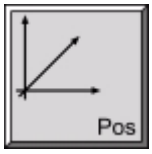
Additional information
The higher the set scan speed, the shorter the dwell time of the laser point. Furthermore, the scan format, i.e. the number of sampling points in a row, needs to be taken into account. The higher the scan format at a constant speed is, the shorter the dwell time of the laser point over a sampling point.

The longer the light point of the laser beam dwells over the individual sampling points of the specimen, the more light is detected by the detector. So using a lower scan speed results in a better signal-to-noise ratio. The disadvantage of a lower scan speed is that the relatively long impact of the light on the specimen can bleach the specimen photochemically, making it unusable. This is especially important with fluorescence applications

Note

If the speed levels 800 or 1000 are set, technical limitations prevent the maximum scan field from being scanned. The system automatically switches to zoom factor 2 or zoom factor 4.

Setting the Z/Y-Position



Function
Use the z/y-Position button to define the horizontal level (z position) or vertical level (y position) of the specimen in which the images are to be recorded. To record an image series using the Series Scan function, define the start and end points of the image series using the z/y-position button and the Begin and End buttons.

see the Series Scan Overview Dialog

Clicking the z/y-Position button opens a dialog window that provides you with two ways of specifying a position value:

- Use the mouse to move the slide on the scale that is displayed on the left in the dialog window. The corresponding position value is shown in the middle of the dialog window.
- Double-click the boldface position value located in the middle of the dialog window. This opens a second dialog window where you can enter an exact value.

The z/y-position can also be set using the corresponding knob on the console.

see Controlling Functions via Control Console

Configuring a Time Series

Function
Click the Time button to open the Time Configuration dialog window for setting up a time series recording. The settings that can be changed in this dialog window depend on the selected scan mode. You can record a line (xt), a horizontal section (xyt), a vertical section (xzt), or a stack of horizontal sections (xyzt), interrupted by a particular time interval, many times in a row.

Note

The Time button is not enabled unless you have selected a scan mode with time dimension using the Mode button.

see Selecting a Scan Mode

Use the following parameters in the dialog window to configure a time series using the xt scan mode.

ΔT	The recording time for a line *(cannot be modified by the user)*
Lines	Number of line recordings
Lines per page	Number of lines per stored page
Pages	Number of stored pages *(calculated automatically)*
Complete Time	Total recording time, i.e. the product of ΔT and number of recordings

Using the Calculate button, you can calculate how many stored pages are needed for a particular number of lines per page.

Use the following parameters in the dialog window to configure a time series using the xyt or xzt scan mode.

ΔT	Recording time for a xy-section or xz-section plus pause interval
Frames	Number of recordings of the xy-section or xz-section
Complete Time	Total recording time, i.e. the product of ΔT and number of recordings

Use the following parameters in the dialog window to configure a time series using the xyzt scan mode:

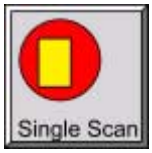
ΔT	Recording time for a stack of xy-sections plus pause interval
Stacks	Number of recordings of the image stack
Complete Time	Total recording time, i.e. the product of ΔT and number of recordings

Each parameter can be calculated in relation to the other parameters. When entering the values, note how the dialog window operates. The entry field you click on will be activated:

- Click on the parameter to be calculated. The corresponding entry field is grayed out.
- Now enter the values for the other parameter.
- Click on Apply to calculate the parameter. By clicking on Reset, the last saved values are displayed.

When you have finished configuring these settings, press the Series Scan button.

Starting a Single Scan



Function
Use the Single Scan button to record a single image from a single optical level in the specimen.

Before recording an image using the single scan function, configure all

required scan parameters using the Continuous Scan function to ensure optimal image quality.

see Starting a Continuous Scan

Typical Applications
The single scan function is designed for recording bleach-sensitive specimens. Use this function in the case of bleach-sensitive specimens not only for image recording, but also as an alternative to the continuous scan function for setting the scan parameters. The single scan function can also be used to check the image section by zooming in on the scan field.

Starting a Continuous Scan

Function
Use the Continuous Scan button to permanently record images from a single optical level in the specimen. This does not generate image series, since the image being generated always replaces the previously generated image.

Press the button a second time to stop the continuous scan.

Additional information
The unit applies the last used scan parameters automatically. You can modify some of the parameters **while** the image is being recorded. Others have to be configured **before** you start the recording:

Before Recording	While Recording
Selecting an Objective	Adjusting the electronic zoom
Selecting a Scan Mode	Setting the Detector
Selecting a unidirectional or bi-directional scan	Adjusting phase

Before Recording	While Recording
Selecting an Objective	Setting the Beam Path
Selecting a Scan Format	Setting the Detection Pinhole
Selecting a Scan Mode	Adjusting the electronic zoom
Selecting a Scan Speed	Setting the Detectors
Selecting a unidirectional or bi-directional scan	Adjusting phase
	Setting the z/y Position

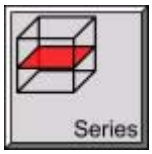
The buttons of the functions that cannot be changed during continuous scanning are disabled and appear gray.

Typical Applications
The continuous scan function is best used to optimize the image quality for the first scan of a specimen. While the specimen is being scanned continuously, you can modify the scan parameters listed above and check the results directly in the image.

Note

With bleach-sensitive specimens it is advisable to restrict the use of the Continuous Scan function to short time intervals. Subjecting the specimen to the continuous light of the laser can destroy the specimen photochemically (optical bleaching), thus making it unusable.

Series Scan Overview Dialog Window



Function
This dialog window lets you define the begin and end points of an image series and trace the recording of the individual sections. The three-dimensional scan area is represented graphically as a cube. Within this cube, a yellow square represents the current z position or y position, a green one represents the begin point and a red one the end point. The corresponding position values are displayed to the right of the cube. Set the begin and end point as follows.

- Use the mouse pointer to drag the yellow square to the level in which the image series is to begin.
  Or click the z/y-Position button to open a dialog window where you can enter the values of the z position or y position.
- Click in the white box for the begin point. The corresponding position value appears and is saved.
- Use the mouse pointer to drag the yellow square to the level in which the image series is to end.
  Or click the z/y-position button to open a dialog window where you can enter the values of the z-position or y-position.
- Click in the white box for the end point. The corresponding position value appears and is saved.
- The entire height of the image stack between the begin and end points is calculated and displayed (total).

Now click the Series Scan button. The dialog window stays open and you can follow the process of the image series being recorded.

Note

The start point and end point can also be defined using the control panel and the separate keys Begin and End.

see Defining the Start Point for a Spatial Series
see Defining the End Point for a Spatial Series
see Setting the z/y Position

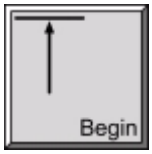
Additional information
The following additional parameters, which have to be set before recording an image series, are specified in the Series Scan Overview dialog window:

- Scan mode (top line to the right of the graphic image)
- Scan format (red digits at the upper edge of the cube)
- Number of optical sections (division between begin and end points)

The red number on the vertical edge of the cube represents maximum travel of the z-actuator that cannot be changed.

see Selecting a Scan Mode
see Selecting a Scan Format
see Defining the Number of Spatial Sections

Defining the Begin Point for a Spatial Series



Function
Use the Begin button to define the begin point of a spatial image series. First, set the exact z-position and/or y-position using the z/y-position button or on the control panel using the corresponding radio button. Then click Begin. This saves the position value for the start point. The end point is set in the same manner.

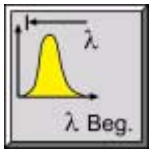
see Setting the z/y Position
see Controlling Functions via Control Panel

Note

You can also conveniently set the begin point and end point of a spatial series in the Series Scan Overview dialog window. Open this dialog window by pressing the Series button (the small button, not the button that starts the Series Scan function).

see The Series Scan Overview Dialog

Defining the Begin Point for a Wavelength Series



Function
For a wavelength series, this function records a stack of individual images, each of which are detected at a specific wavelength, from a single, optical level. Use the Lambda Scan Begin button to define the wavelength at which recording should begin:

Note

The Lambda Scan Begin button is only active if you used the Mode button to select a scanning mode with the wavelength dimension.

see Selecting a Scan Mode

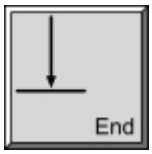
- Press Beam to open the Beam Path Setting dialog window.
- Activate a detector by clicking its corresponding check box. A cast shadow now links the activated detector to the corresponding slide bar on the scale of the spectrum.
- Double-click this slide bar to open the Range Properties dialog window, where you can specify the end point of the series.
- Drag the slide bar on the scale of the spectrum to the desired start position. The left edge of the slide bar represents the wavelength at which the recording is to begin.
- Click Lambda Scan Begin to save the value.

The end point of a wavelength series is set in the same manner. In addition, you also have to set the number of wavelength steps.

see Defining the End Point for a Wavelength Series
see Setting the Number of Wavelength Steps

Typical Applications
A wavelength series can be applied to determine the maximum emission of a fluorochrome. This is useful because the Stokes shift of the emission curve of a fluorochrome is dependent on each specimen that is applied. This then allows you to set the detection range precisely for a specific application.

Defining the End Point for a Spatial Series



Function
Use the End button to define the end point of a spatial image series. First, set the exact z position or y position with the z/y Position button or with the corresponding rotary knob on the control panel. Then click End. This saves the position value for the end point. The begin point is set in the same manner.

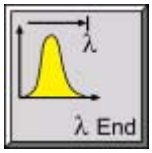
see Setting the z/y Position
see Controlling Functions via Control Panel

Note

You can also define the begin and end points for a spatial series in the Series Scan Overview dialog window. Open this dialog window by pressing the Series button (the small button, not the button that starts the Series Scan function).

see The Series Scan Overview Dialog

Defining the End Point for a Wavelength Series



Function
For a wavelength series, this function records a stack of individual images, each of which are detected at a specific wavelength, from a single, optical level. Use the Lambda Scan End button to define the wavelength at which recording should end:

Note

The Lambda Scan End button is only active if you used the Mode button to select a scanning mode with the wavelength dimension.

see Selecting a Scan Mode

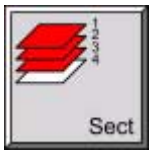
- Press Beam to open the Beam Path Setting dialog window.
- Activate a detector by clicking its corresponding check box. A cast shadow now links the activated detector to the corresponding slide bar on the scale of the spectrum.
- Double-click this slide bar to open the Range Properties dialog window, where you can specify the end point of the series.
- Drag the slide bar on the scale of the spectrum to the desired end position. The right edge of the slide bar represents the wavelength at which the recording is to end.
- Click Lambda Scan End to save the value.

The begin point of a wavelength series is set in the same manner. In addition, you also have to set the number of wavelength steps.

see Defining the Begin Point for a Wavelength Series
see Setting the Number of Wavelength Steps

Typical Applications
A wavelength series can be applied to determine the maximum emission of a fluorochrome. This is useful because the Stokes shift of the emission curve of a fluorochrome is dependent on each specimen that is applied. This then allows you to set the detection range precisely for a specific application.

Defining the Number of Spatial Sections



Function
Clicking the Sections button opens a dialog window that you can use to select the number of horizontal xy-sections and vertical xz-sections for recording an image series. If you want to specify a number of sections that is not listed, click the Others option. This opens the Z/Y Configuration dialog window, which contains the following data:

Parameter	Description
Image Dim. z/y (mm)	The height of the entire image stack between the begin and end points of the image series
# Sections	The number of configured sections
Step Size (mm)	The step size, i.e. the distance between two sections

In this dialog window, you can enter any value for the number of sections and the section width. The height of the image stack cannot be changed, as this parameter is determined by the start and end point of the image series. As the section width always has to be a multiple of the minimum section width of the z-setting drive, certain combinations of values are only possible if either the height of the image stack or the number of sections is adapted. Depending on which of the two Calculate buttons you click, one of the two parameters is not changed.

If you want to calculate the number of sections with the priority of leaving the height of the image stack unchanged where possible:

- Enter the desired step size in the Step Size field.
- Then click the Calculate button next to the Step Size field.

If you want to calculate the number of sections with the priority of leaving the number of sections unchanged where possible:

- Enter the desired step size in the Step Size field.
- Then click the Calculate button next to the # Sections field.

If you want to calculate the step width with the priority of leaving the height of the image stack unchanged where possible:

- Enter the number of desired sections (whole numbers only) in the # Sections field.
- Then click the Calculate button next to the # Sections field.

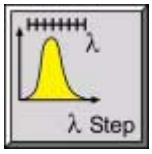
If you want to calculate the step width with the priority of leaving the number of sections unchanged where possible:

- Enter the number of desired sections (whole numbers only) in the # Sections field.
- Then click the Calculate button next to the Step Size field.

If you click the Reset button, the last shown values are displayed.

see Starting a Series Scan
see Series Scan Overview dialog window

Setting the Number of Wavelength Steps



Function
For a wavelength series, this function records a stack of individual images, each of which are detected at a specific wavelength, from a single, optical level. The images are recorded within a wavelength range, which is limited by the begin and end points. Use the Lambda Steps button to define the number of recordings that are to take place within this range.

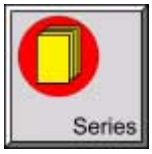
Note

The Lambda Steps button is only active if you used the Mode button to select a scanning mode with the wavelength dimension.

see Selecting a Scan Mode
see Defining the Begin Point for a Wavelength Series
see Defining the End Point for a Wavelength Series

Typical Applications
A wavelength series can be applied to determine the maximum emission of a fluorochrome. This is useful because the Stokes shift of the emission curve of a fluorochrome is dependent on each specimen that is applied. This then allows you to set the detection range precisely for a specific application.

Starting a Series Scan



Function
Use the Series Scan button to create an image series. This creates a multidimensional image data block of the specimen. The available dimensions for recording an image series are the three directions in space (x, y, z) as well as the dimensions of time (t) and wavelength (λ). This allows you to record a three-dimensional, spatial image stack consisting of xy or xz-sections with the additional factor of time or wavelength.

Note

Before recording an image series, configure all required scan parameters using the Continuous Scan function to ensure optimal image quality.

see Starting a Continuous Scan

Before recording a spatial image series, configure the following settings:

- Select the scan mode using the Mode button.
  see Selecting a Scan Mode
- Select the scan format using the Size button.
  see Selecting a Scan Format
- Set the desired z-position or y-position using the z/y-position button or the corresponding knob on the console.
  see Setting the z/y Position
  see Controlling Functions Using the Console
- Set and store the begin point for an image series using the Begin button or in the Series Scan Overview dialog window.
  see Defining the Begin Point for a Spatial Series
- Set the desired z-position or y-position using the z/y-position button or the corresponding knob on the console.
  see Setting the z/y Position
  see Controlling Functions Using the Console
- Set and store the end point for an image series using the End button or in the Series Scan Overview dialog window.
  see Defining the End Point for a Spatial Series
- Select the number of spatial sections using the Sections button.
  see Defining the Number of Spatial Sections

When you have finished configuring these settings, press the Series Scan button.
You can track the recording process of the image stack in the Series Scan Overview dialog window.

Before recording a time series, configure the following settings:

- Select a scan mode with time dimension using the Mode button.
  see Selecting a Scan Mode
- Select the scan format using the Size button.
  see Selecting a Scan Format
- Set the desired z-position or y-position using the z/y-position button or the corresponding knob on the console.
  see Setting the z/y Position
  see Controlling Functions Using the Console
- Set the number of recordings, the pause interval between the recordings, and the complete editing time.
  see Configuring a Time Series

Before recording a wavelength series, configure the following settings:

- Select a scan mode with wavelength dimension using the Mode button.
  see Selecting a Scan Mode
- Select the scan format using the Size button.
  see Selecting a Scan Format
- Define the wavelength at which you want the wavelength series to begin.
  see Defining the Begin Point for a Wavelength Series
- Define the wavelength at which you want the wavelength series to end.

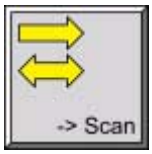
see Defining the End Point for a Wavelength Series

- Select the desired number of recordings between the begin and end points of the wavelength series.

see Setting the Number of Wavelength Steps

When you have finished configuring these settings, press the Series Scan button.

Selecting a unidirectional or bi-directional scan



Function
If you click the Unidirectional/ Bi-directional Scan button, bi-directional scan mode is enabled. If this button is not clicked, unidirectional scan mode is automatically set.

In unidirectional scan mode, each line is scanned from left to right. No data is recorded while the laser beam is being guided to the starting point of the next line. In bi-directional scan mode, the first line is scanned from left to right and the second from right to left. In other words, the return run, or flyback, of the laser beam is implemented for recording data too, thus increasing the scan speed when using bi-directional scan mode.

If bi-directional scan mode is active, you can double the scan speed using the Speed button:

Unidirectional	Bi-directional	
200	Currently not available	Image lines per second
400	800	Image lines per second
800	1600	Image lines per second
1000	2000	Image lines per second

see Selecting a Scan Speed

In order to accurately align the pixels of the forward sweep and flyback, the phase between the forward sweep and the flyback can be adjusted. Use the Phase button or the appropriate knob on the control panel.

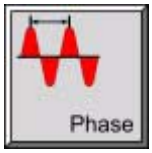
see Setting the Phase
see Controlling Functions using the Control Panel

Note

If the speed levels 800 or 1000 are set, technical limitations prevent the maximum scan field from being scanned. The system automatically switches to zoom factor 2 or zoom factor 4.

see Electronic Zoom

Adjusting phase



Function
During bi-directional image recording, there may be a phase displacement between the scanning beams moving forward and backwards. Click on the Phase button to open a dialog window that allows you to correct the displacement:

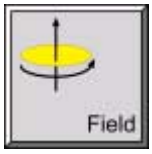
- Use the mouse pointer to move the slider on the scale until the pixel displacement disappears from the image.

  It is also possible to adjust the phase with the respective knob on the control panel.

see Controlling Functions using the Control Panel

Additional information
Before delivery of the instrument, the factory performs a phase adjustment for each zoom value and enters the corresponding values. Due to the temperature dependency of the mechanics and electronics, there may be a slight deviation from these default values during operation. Use this function to readjust these values.

Turning the Scan Field



Function
Use the Scan Field Rotation button to rotate the scan field. This neither rotates the specimen nor the direction of the scanner, but rather the intermediate microscopic image.

The angle of rotation is infinitely variable between -10 and +100 degrees. The original position (0 degrees) is based on a predefined reference position in the scanning head. When the unit is switched on, it automatically relocates to this reference position.

Typical Applications
This function is used to align textures on surfaces or long stretched-out biological structures relative to the viewer.

Recording an Image of a Line Using the Averaging Method

Function
The Line Average function launches an averaging method for the image recording. In this case, each single line is scanned several times. For every sampling point, the arithmetical average is calculated from the repeatedly measured intensity values in one line and represented in the result image. The next line of the specimen will not be scanned until after the set number of averaging steps has completed. The method used here determines a consecutive average. This means that every line recorded after the first line is averaged with the previously displayed line and is displayed in the result image (dynamic average).

Clicking the Line Average button opens a dialog window that you can use to set how often a line is to be scanned. You can select from 1 to 8 scan repeats.

Using a parallel function, it is possible to record complete images using the averaging method.

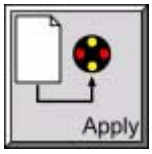
📖 see Recording an Image Using the Averaging Method

Typical Applications
This function is used predominantly for recording live specimens.

Note

Recording an image using the averaging method is not recommended for bleach-sensitive specimens. Repeated recordings and the resulting length of subjection to light can lead to the destruction of the specimen.

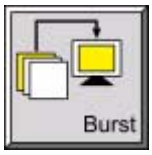
Applying the Parameter Setting of an Experiment



Function
Click the Apply button to use the hardware settings of a previous experiment for recording a new experiment. This lets you configure the settings for new image recordings with a single click with the scan parameters that have been optimally configured for a previous application.

- Activate the image data block that is configured with the settings you want to apply to the new experiment.
- Click the Apply button.

Recording an Image Using Burst Mode



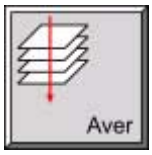
Function
If the workstation must process large amounts of data during the recording of images, it may lead to a delay of the scanning process. This is because the recorded image is first displayed on the monitor before the scanner can scan additional images. The Burst function allows for decoupling the scan process of the laser and the refreshing of image data on the screen. For this purpose, the transmission of image data from the program memory to the monitor is delayed, but the scanning process is not. By clicking on the Burst button, you can select operating modes with different delay times:

Mode	Description
No Burst	Image data are recorded and simultaneously and continuously displayed on the monitor.
Frame Burst	Image data are not displayed on the monitor until a single image has been recorded.
Complete Burst	Image data are not displayed on the monitor until an image series has been recorded.
Automatic	The software automatically sets the optimum-operating mode for a specific application.

Note

The following parameters can increase the amount of data to be processed to the extent that it will delay the display on the monitor: Scanning format, scanning speed, bi-directional scan, number of active detection channels, calculation of an overlay image, and size of the Viewer window.

Recording an image using the Averaging Method



Function
The Average function launches an averaging method for the image recording. In this case, every individual image, i.e. every xy-section or xz-section, is scanned several times. For every sampling point, the arithmetical average is calculated from the repeatedly measured intensity

values and represented in the result image. The method used here determines a consecutive average. This means every image recorded after the first image is calculated with the results of the previously displayed image and is displayed in the result image (dynamic average).

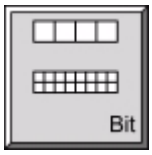
Clicking the Average button opens a dialog window that you can use to set how often a section is to be scanned. You can select from 1 to 64 scan repeats.

Typical Applications
Recording an image using the averaging method is primarily useful for suppressing noise. In fluorescence microscopy, weak fluorescent specimens result in little light reaching the detector. The resulting low photon count leads to noisy images. In such cases, you can improve the signal-to-noise ratio by carrying out multiple recordings and determining averages of the image.

Note

Recording an image using the averaging method is not recommended for bleach-sensitive specimens. Repeated recordings and the resulting length of subjection to light can lead to the destruction of the specimen.

Image Recording With a Digital Resolution of 8 Bits or 12 Bits



Function
This function reads in the analog intensity values measured by the detector by means of an A/D converter, either as 8 bit signal or 12 bit signal.

Additional information
While digitizing the analog intensity signal with a digital resolution of 8 bit, 256 different intensity values can be displayed. Taking the natural statistical variations of the typical intensity value range of fluorescence specimen into account, a digitization with 8 bit is completely sufficient in most cases for an image recording without loss of information. For specimens with a higher intensity dynamics (e.g., for specimen with very intensity-weak and intensity-strong areas), an image recording with 12 bit digitization is recommended. In a 12-bit digitization, 4096 different intensity values can be resolved.

Typical Applications
For image recording with very high intensity dynamics (mostly material specimens with high reflectivity of the surface and occurrences of very dark areas at the same time).

Setting the UV Lens Wheel

Function
For each objective, this function reduces the focus offset between visible and UV excitation light to a value below the optical resolving capacity. In order to achieve this, a suitable correction lens is brought into the beam path for each objective suitable for use with UV excitation light. To use this corrective feature, proceed as follows:

- Click the Objective button (standard procedure in Acquire step).
  see Selecting an Objective
- Select a UV-capable objective from the list (see indication on list).

In this case, the corrective lens for the selected objective is automatically moved into the beam path.

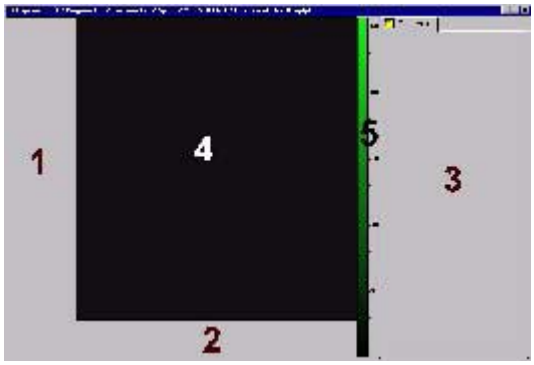
Note

The possible occurrence of a focus offset between visible light and UV light has physical reasons and is not a malfunction of the device.

Other Requirements
In order to achieve an automatic correction of the physically based color length fault between visible and UV light, it is not sufficient to manually rotate the objective into the beam path.

Data Viewing

The Viewer Window



In the default setting, the Viewer window consists of three important areas. The image window (4), in which the recorded images are displayed, is located in the middle. Button pads (1) and (2) can be arranged to the left and below the image window and the Experiment legend (3) to the right of it. It is possible to select other default settings of the Viewer window and to save user-defined configurations as a template.

see Saving Viewer Window Viewer as a Template

Button pads (1) and (2)
By default, the button pad to the left of the image window contains the buttons for image viewing functions and the button pad below the image window contains the buttons used for scrolling through the individual images of an image series. Both button pads can be moved within the Viewer window or removed from it as separate windows. To do so, click on the double-lined edge of the button pad and drag it while keeping the mouse button depressed to the desired position.

Image window (4)
If you place the mouse pointer at any position within the image window and then press the right mouse button, a context menu containing the following commands opens:

Command	Function		
Send to	Experiment	Selection (raw)	This copies the raw data of a selected area in the image window as a new image in the current experiment. The new image can be processed.
		Selection (snapshot)	This creates a new image in the current experiment from the snapshot of a selected area in the image window. The new image can no longer be processed.
		All (snapshot)	This creates a new image in the current experiment from the snapshot of the entire, current image window. The new image can no longer be processed.
	Printer	Selection	This prints the area selected in the image window.
		All	This prints the entire, current image window.
Left buttons	This shows or hides the button pad to the left of the image window.		
Bottom buttons	This shows or hides the button pad located below the image window.		
LUT	This shows or hides the color look-up tables of the current image.		
Legend	This shows or hides the Experiment legend.		
Full screen	This enlarges the Viewer window to full screen.		
Viewer Options	This opens the Viewer Options dialog window.		

The **color look-up tables (5)** are displayed as color bars to the right of the image window. If you hold the mouse pointer over a color bar, grab points appear at the top and bottom of the color bar. You can use these grab points to limit the current color look-up table to a specific intensity range and to load a second color look-up table. This allows you to increase the contrast of the image graphically.

- Drag the top grab point down or the bottom grab point up.
- Double-click the area above or below the corresponding grab point.
- The Select LUT's dialog window opens so that you can select a second color look-up table.
- The upper and lower intensity ranges are represented in the colors of the second color look-up table.

see Selecting Color Look-Up Tables (LUT)

The Experiment (3) Legend
Various image parameters of an image recording are registered in the Experiment legend. To select the parameters that you want displayed, click any area of the legend. This opens a list of all available entries. Then select the desired entry. If you hold the mouse pointer over any area within the Experiment legend and press the right mouse button, a context menu containing the following commands opens:

Command	Function	
Experiment	Edit	This opens the Edit Legend dialog window, where you can specify a (Title) for the legend, specify the (Number of legend entries) or (Clear all entries).
	Activate	This displays the Experiment legend in the Viewer window.
	Remove	This deletes the current Experiment legend.
Add Experiment tab	This creates a new Experiment legend in the Viewer window.	

The Hardware Legend
The hardware settings for image recording are registered in the Hardware legend. To open the legend, select the Hardware Legend option from the View menu. To select the entries that you want displayed in the legend, click the Edit button. This opens the Edit Legend Entries dialog window:

- The Available Entries list box shows all available entries. Select the entries that you want displayed in the legend. Then click Add to carry the selected entries over into the Show entries list box.
- The Show entries list box contains the entries that are to be displayed in the legend. Use the Remove button to remove entries from being displayed in the legend.
- Use the Move up and Move down buttons to shift one or more than one entry up or down in the list.
- You can use the Edit grid color and Edit background color buttons to modify the color of the frame and background of the legend.

The Hardware legend is aligned automatically along the right edge of the user interface. You can change however the size and position of the legend as desired:

- To change the width of the legend, use the mouse pointer to drag the edge to the position you desire.
- To change the position of the legend, double-click on the double-lined edge of the legend or click the 5 icon once. This releases the legend from the user interface, turning it into a window. Now you can drag the legend to any position by pressing and holding the left mouse button.

Note

If the legend has been enlarged to cover the entire width of the user interface, you first have to shrink the height of it in order to drag it back to the edge of the user interface.

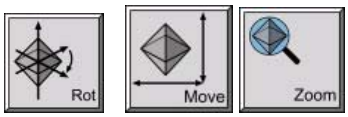
Option New Window
You can open a second Viewer window containing the same image as the currently open Viewer window by clicking the New Window option in the Window menu. This command does not create a new experiment, but rather opens a second view of the current image data. This feature is useful for viewing different representations of the same image data at the same time. Sequential numbers are added behind the file extension to distinguish between the resulting copies of the current Viewer window.

Viewer Options

Viewer Options Dialog Window, 3D Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

The tabs of the 3D icon can be used to perform optional settings for the following functions:



see Rotating the 3D View
see Moving the 3D View
see Zooming the 3D View

The **Navigation tab** displays the numerical values of the actions that are carried out using the Rotate, Move and Zoom buttons (and the mouse pointer).

Use the **Rotation field** to tilt a 3D view in all three spatial directions by modifying the angular degrees of the three axes. The 3D view is rotated around a fixed point, which is located in the center of the image. To best understand the rotation function, modify the angle of one axis at a time, while leaving the other two axes set to 0.

Rotation	Function
X from 0° to 45°	The 3D view is rotated 45° around the fixed point in the direction of the negative z-axis.
Y from 0° to 45°	The 3D view is rotated 45° around the fixed point in the direction of the negative y-axis.
Z from 0° to 45°	The 3D view is rotated 45° around the fixed point in the direction of the negative y-axis.

Use the **Translation field** to shift a 3D view to the right or left, up or down and enlarge or shrink it by modifying the coordinate values. (Users of all regional versions should be sure to use the dot as the decimal placeholder.)

Translation	Function
X	Positive values shift the 3D view to the right, negative to the left.
Y	Positive values enlarge the 3D view, negative shrink it.
Z	Positive values shift the 3D view up, negative down.

Use the **Predefined field** to change the viewing perspective of the image to Top view or Side view by clicking these buttons respectively.

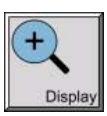
In the **Display tab** click on one of the listed commands to show or hide the corresponding graphic element of the 3D view in the Viewer window:

Graphic Elements	Function
Show LUT	Show the selected color look-up table on the z-axis.
Show scale	Show the scale.
Show bounding box	Show the bounding box, which limits the measurement volume.
Show axes	Show the coordinate axes.
Show data during 3D motion	Show the image data while the 3D view is being rotated, shifted, enlarged or shrunken.

Viewer Options Dialog Window, Display Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

The tabs of the Display icon can be used to perform optional settings for the following functions:



see Starting and Ending a Film

On the **Settings tab**, use the graphic zoom to enlarge or reduce the image displayed in the Viewer window:

n-1	Displaying n pixel as 1 pixel in the resulting image reduces the image.
Automatic	The image is displayed in the original image format.
1-n	Displaying 1 pixel as n pixel in the resulting image enlarges the image.

Note

The Leica Confocal Software provides three different zoom functions: the graphic zoom, the 3D zoom and the electronic zoom.

see Zooming the 3D View
see Electronic Zoom

Click on one of the selection points on the same tab to hide or display the respective graphic element in the Viewer window:

Coordinates	The z position of the current image is displayed.
Scale	A measurement bar with length indicators is displayed.
Grid	A grid is placed over the current image.

The length of the measurement bar and the grid width are calculated dependent on the objective, the electronic zoom and the beam divergence.

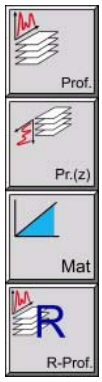
You can use the **Movie tab** to set the speed for running the film sequence of an image series. You can choose speeds from 6 images per minute to 25 images per second.

- Use the mouse to drag the slide on the scale to the desired value.
- Select the Ping-Pong mode if the film sequence is to run from the first to the last image and then in reverse order back to the first image. If this mode is not selected, the film sequence always starts with the first image again.

Viewer Options Dialog Window, Charts Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

The tab of the Charts icon can be used to perform optional settings for the following functions:



see Measuring a Profile Along a Line Segment

see Measuring a Profile Within a Region of Interest (ROI)

see Measuring Surfaces and Volumes

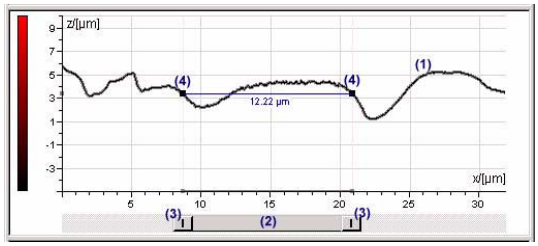
see Measuring Roughness Along a Line Segment

The options in **Charts tab** can only be selected if one of these buttons was used earlier to activate a quantification function.

Activating the two-point measurement

- Clink on 2 Point in the **Measurement field** to activate the two-point measurement.

Each of the buttons listed above opens a window that displays a measuring curve and statistical values.
If the two-point measurement is selected, the window shows a measurement slider (2) with two measurement points (3) below the measurement curve (1). The position of the two measurement points in the profile curve is symbolized by small black squares (4). This allows for very precise settings of the range to be evaluated within the measurement. In addition, the statistics shown below the measurement curve is expanded by the following measurement values: position of measurement points, distance of measurement points, measurement value at the measurement points, difference of the measured variable between the measurement points.



Changing the position and length of the measurement slider

- To change the position, move the mouse over the slider, click and hold the left mouse button and drag it to the desired position.
- To change the length of the slider, grasp it at one end with the mouse and enlarge or reduce it while pressing the left mouse button.

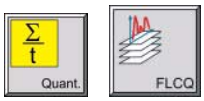
Changing the scaling of the measurement curve
By default, the scaling of measurement curves is automatically adapted by the software to the intensity value range that is present in an image recording. However, the scaling can also be changed manually:

- Deselect the Auto check box to deactivate automatic scaling.
- You can now redefine the upper and lower limit value of the value range separately for each measurement curve (each detection channel).
- Check the results of the changed scaling directly in the window.

Viewer Options Dialog Window, Online Measure Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

In the tab of the Online Measure icon, you can make optional settings for the following functions:



The slider on this function can be used to set the number of optical sections for which the measured values of an online measurement are displayed graphically. The developing trend in an experiment can be seen from the online measurement graph.

Note

The trend data only exists in temporary form and it cannot be saved. The temporarily saved data can however be exported via the context-sensitive menu (right mouse button) or copied into an annotation sheet.

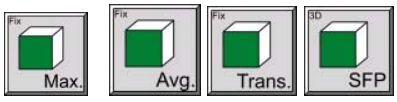
see Exporting Quantification Data
see Copying Quantification Data to the Annotation Sheet

Typical Applications
Quantitative evaluation of data records

Viewer Options Dialog Window, Projections Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

In the tabs of the Projections icon, you can make optional settings for the following functions:



see Maximum Projection of an Image Stack With Invariable Projection Angle
see Average Projection of an Image Stack With Invariable Projection Angle
see Transparent Projection of an Image Stack With Invariable Projection Angle
see Creating an SFP Projection of an Image Stack

The **Projections** tab contains various options for generating a projection image. Select whether the intensity maximums, the arithmetical average or a weighted average of the intensities are to be displayed in the projection image. Vary the weight factor for the transparent projection. Set a threshold value or scaling factor for the displayed intensities.

Type	**Function**
Maximum projection	From each of the examined columns of sampling points, the sampling point with the highest intensity is displayed in the two-dimensional projection image as the representative of all values within the column.
Average projection	From each of the examined columns of sampling points, the arithmetical average of the intensities measured in the column is displayed in the projection image.
Transparent projection	From each of the examined columns of sampling points, a weighted average of the intensities measured in the column is displayed in the projection image.

Transparent factor	**Function** (enabled only for transparent projection)
Drag the slide on the scale.	The higher transparent factor a (0 < a < 1) setting, the stronger the intensity values flow from the lower levels in the image stack into the projection image.

Threshold	**Function** (enabled only for average and transparent projection)
Drag the slide on the scale.	Intensities that are below the specified threshold value are ignored in the calculation of the projection image.

Scaling	Function (enabled only for average and transparent projection)
Rescale to maximum	The measured intensity values are scaled to the maximum possible intensity values.

The **SFP tab** contains various options for generating an SFP projection image.

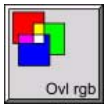
see Principles and Types of Projections

Field	Function
Light Direction	Here, enter a coordinate for x and y for the projection angle of the SFP projection. This angle symbolizes the angle of incidence of the laser beam onto the specimen. Click on the Apply button to create the projection with the new angle.
Absorption	You use the slider to vary the absorption coefficient a. The higher this is set, the smaller the values calculated in the projection image. If the coefficient is set as low, many of the pixels in the projection image reach the maximum value, making it increasingly difficult to distinguish between the structures.
Threshold	Use the slider to define a threshold value. The intensity values below this value are not taken into account for the creation of the projection image.
Rescale to maximum	The intensity values of the image are scaled to the maximum possible intensity values. This ensures that the calculated gray values of the projection image remain within the value range of, for example, 0 to 255 at 8 bits. This allows for brightening dark images.

Viewer Options Dialog Window, Overlay Icon

Structure of the Experiment Overview window
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

The tab of the Overlay icon can be used to perform optional settings for the following functions:



see Viewing an Overlay Image

On the **Overlay tab** you can select among three types of creating an overlay image from raw data and the corresponding color look-up tables:

Color Merging	Function
True Color (add RGBs)	The color values of the pixels in the original images are averaged and displayed in the overlay image.
True Color (add RGBs) with dynamic adjustment	The color values of the pixels in the original images are averaged, scaled to the maximum possible color values and displayed in the overlay image.
Fast (bit wise "OR" RGB)	The color values of the pixels in the original images are mixed using a fast bit-by-bit calculation process and displayed in the overlay image.

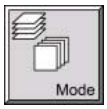
see Boolean Operations

Coloring	Function
Only Red/ Green/ Blue	The color look-up tables Red, Green and Blue are always used to create the overlay image, independent of the current color look-up tables of the original images.

Viewer Options Dialog Window, Scan Progress Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

In the tab of the Scan Progress icon, you can make optional settings for the following functions:



see Selecting a Scan Mode

The currently set scan mode is shown in the **Scan Progress** tab. The status of an image recording can also be checked on the basis of the progress display.

Viewer Options Dialog Window, Surface View Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options

window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

In the tab of the Surface View icon, you can make optional settings for the following functions:

see Creating the 3D View

In the **Visualization tab**, you can select whether the 3D view is to be generated as Surface, Wireframe or Isolines and specify the perspective in which the 3D view is displayed:

Render Mode	Function
Surface	The blank spaces between the pixels are filled in with surface.
Wireframe	All pixels are linked with lines, while the blank spaces remain free.
Isolines	Pixels that correspond to values of the same intensity are enclosed in a curve.

Projection Type	Function
Perspective	The 3D view is displayed in central perspective.
Parallel	The 3D view is displayed in parallel perspective.

The **Stretch height (factor)** field offers the ability to vary the scaling factor in the z-direction, thus stretching or shrinking the height of the 3D view.

Use the **Downsample rate** field to reduce the density of data of the 3D view to increase the speed of image processing. At a pixel density of 1:1, all measured intensity values are displayed in the image. At a pixel density of 1:2, only every second measured intensity value is used in the surface image.

Use the **Isoline interval** field to define a distance for separating the individual isolines in mm. This allows you to limit the number of isolines in the 3D view.

Use the **Isoline detail level** field to enter a limiting value, specifying that only isolines of a particular length are to be displayed in the 3D view. This limits the displayed isolines to those, which correspond to an intensity value that has a specific frequency of occurrence.

Viewer Options Dialog Window, Surface Measure Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

In the tab of the Surface Measure icon, you can make optional settings for the following functions:

see Measuring Roughness Along a Line Segment

In the **Leveling** field, there are options for an adaptation function to bring measurement curves of a roughness profile that show a trend into a horizontal position. For this purpose, a linear interpolation function (polynom) is fitted to the measurement curve.

see Image Tool Dialog Window, Basic Button
see Adaptation Function for Correction of Trends

None	No adaptation function is applied to the measurement curve.
Automatic linear	The measurement curve is brought into a horizontal position using a linear adaptation function.
Interactive level	The position of the measurement curve is corrected using the linear adaptation function in such a way that the line segment between the two measurement points is on the horizontal plane.
Freeze	The newly positioned measurement curve is locked so that the item of the measurement points can be changed again without interactively modifying the measurement curve.

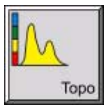
The **Multipoint Measurement** field provides the possibility of saving the two measurement points as well as the difference in height between the measurement points.

- Click on the Remember button to save the values. The Clear button deletes them again.

Viewer Options Dialog Window, Surface Calculation Icon

Function
To open the Viewer Options window, select the Experiment Overview option in the View menu.
The Experiment Overview window appears on the left side of the user interface. The top part of the viewer window displays the recorded images in a directory tree. The bottom part displays the Viewer Options window. This dialog window is used to perform basic settings for different software functions. The left side shows the icons corresponding to the functions and the right side shows the related tabs. When you open the dialog, it contains the icons of the functions that you are currently using. Click Show all to view all icons.

In the tab of the Surface Calculation icon, you can make optional settings for the following functions:



see Creating a Topographical Image

The **Topography tab** contains various options for generating a topographical image. Select whether the intensity maximums or centers of mass of the intensities are to be interpreted as height in the topographical image. Specify the type of visualization for height in the topographical image and set a threshold value for the displayed intensities.

Surface Reconstruction	Function
Search maximum intensity	From each of the examined columns of sampling points, the sampling point with the highest intensity is displayed in the two-dimensional topographical image as the representative of all values within the column.
Calculate center of mass of intensities	From each of the examined columns of sampling points, the center of mass of the surface, which is restricted by the curve of the measured intensity values, is displayed in the two-dimensional topographical image.

Topography Processing	Function
Invert height	The column of sampling points is examined in reversed direction. This reverses the height information. A negative of the topographical image is displayed.
Level	The horizontal position of a recording is corrected using a linear interpolation function (polynom). see Image Tool Dialog Window, Basic Button

Threshold	Function
Drag the slider on the scale or enter a numerical value.	Intensities that are below the specified threshold value are ignored in the calculation of the topographical image.

Image Tool

Image Tool Dialog Window

Schematic Structure of the Dialog Window
The numerous functions of the Image Tool dialog window are arranged on a number of levels. The gray buttons 3D, Basic, Amplitude, Materials and Multicolor on the left side of the dialog window represent the first level of the hierarchy. Each of these buttons has a group of icons that appear below the selected button. The icons are the next level on which functions are grouped. Each icon opens different tabs and fields that are displayed on the right side in the dialog window and arrange the functions on the lowest level.

The following elements can be found in each tab:

- In the Source Image drop-down list at the top of the dialog window, select the image data record to be processed.
- If you click on the Use Selected Image check box, the image data record currently displayed in the Viewer window is used for the calculation.
- If you click on the Show button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the preview window in the dialog window.
- If you click on the Apply button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the Viewer window and created as a new file in the current experiment.
- Click on the button with the arrow symbols to show the preview window on the right edge of the dialog window or to hide it. The projections triggered using Show are displayed temporarily in this preview window.
- The Close button closes the dialog window.

see Creating an Experiment

Overview of the Function Groups Created in the Image Tool Dialog Window

Button	Icon	Tab	Description of Function
3D	Projections & Animations	Projections Rotation Stereo View	Creating projection images along a variable projection axis Running Projection Images as 3D Rotation Animation Creating red-green stereo views
	Orthogonal Projections	_____	Creating projection images along the orthogonal axis Creating Projection Images from Different Detection Channels
	SFP (Simulated Fluorescence Process)	SFP Rotation Stereo View	Creating SFP Projection Images Running SFP projection images as rotation animation

	Filter (Linear)	_____	Creating red-green stereo views Applying lowpass and highpass filters to an image data record

see Image Tool Dialog Window, 3D Button

Button	Icon	Tab	Description of Function
Basic	Leveling	_____	Adapting an interpolation function to a data set to correct trend curves
	Merging	Voxel x / y / z / ch / t / λ	Combining several images or data sets to a new image or data set
	Stereo	Projections Stereo View	Creating projection images along the orthogonal axis Creating red-green stereo views
	Image Separation	_____	Cutting single images or image sections from a data record

see Image Tool Dialog Window, Basic Button

Button	Icon	Tab	Description of Function
Amplitude	Arithmetic With Two Images	_____	Running Arithmetical Operations with Images and/or Masks
	Arithmetic With Constant	_____	Running Arithmetical Operations with Images and a Constant
	Bit Converter	_____	Conversion of 8-bit images to 12-bit images and vice versa
	Brightness and Contrast	_____	Setting image brightness and contrast
	Gamma Correction	_____	Setting the Contrast Transfer Function (Gamma Curve)

see Image Tool Dialog Window, Amplitude Button

Button	Icon	Tab	Description of Function
Materials	Filter (Linear)	_____	Applying low-pass and high-pass filters to images

see Image Tool Dialog Window, Materials Button

Button	Icon	Tab	Description of Function
Multicolor	Cross Talk	_____	Removing cross talk between detection channels

Image Tool Dialog Window, 3D Button (optional)

Schematic Structure of the Dialog Window
The numerous functions of the Image Tool dialog window are arranged on a number of levels. The gray buttons 3D, Basic, Amplitude, Materials and Multicolor on the left side of the dialog window represent the first level of the hierarchy. Each of these buttons has a group of icons that appear below the selected button. The icons are the next level on which functions are grouped. Each icon opens different tabs and fields that are displayed on the right side in the dialog window and arrange the functions on the lowest level.

The following elements can be found in each tab:

- In the Source Image drop-down list at the top of the dialog window, select the image data record to be processed.
- If you click on the Use Selected Image check box, the image data record currently displayed in the Viewer window is used for the calculation.
- If you click on the Show button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the preview window in the dialog window.
- If you click on the Apply button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the Viewer window and created as a new file in the current experiment.
- Click on the button with the arrow symbols to show the preview window on the right edge of the dialog window or to hide it. The projections triggered using Show are displayed temporarily in this preview window.
- The Close button closes the dialog window.

Projections and Animations Icon	Creating Projection Images Along a Definable Projection Axis Running Projection Images as 3D Rotation Animation Creating Red-Green Stereo Views

Creating Projection Images Along a Definable Projection Axis

- In the **Projections** tab, select one of the three projection types: maximum projection, average projection, and transparent projection.
  see Principles and Types of Projections
- Click on Rescale in the Options field to standardize the intensity values of the image to the maximum possible intensity values when creating the projection image. This ensures that the calculated gray values of the projection image remain within the value range of, for example, 0 to 255 at 8 bits. This allows for brightening dark images.
- If you click on Invert in the Options field, the projection axis is mirrored. The calculation of the projection image is thus in the opposing direction. This function makes sense above all in the case of inverse microscopes to match the calculation of the projection image to the inverse beam path of laser light.
- The Transparent Factor field is only active if the transparent projection was selected. You use the slider to vary the transparent factor a. The higher it

is set, the stronger the intensity values flow from the lower levels in the image stack into the projection image. In general, this includes more information in the projection image.
see Principles and Types of Projections

- Move the slider in the Threshold field to define a threshold value. The intensity values below this value are not taken into account for the creation of the projection image.
- To set the projection axis, click on the Rotation tab (see below for description).
- Click on the Show button to obtain a preview of the projection images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the projection as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

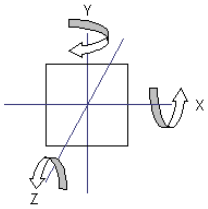
Note

The three projection types can also be enabled directly using the corresponding keys (3D Proj / 3D Max / 3D Avg / 3D Trans). If you activate the function using the button, the projection image will just be a temporary display on the monitor. You can undo this using the Original key.

see Projection of an Image Stack With Variable Projection Axis
see Maximum Projection of an Image Stack With Variable Projection Axis
see Average Projection of an Image Stack With Variable Projection Axis
see Transparent Projection of an Image Stack With Variable Projection Axis

Setting the Projection Axis and Running Projection Images as a 3-dimensional Rotation Animation

- In the **Rotation** tab, set the desired projection axis by entering a value for Z, Y and X. With these three values, you rotate the image stack into a certain position and thus define the angle at which the image stack is projected. The projection axis here is the viewing angle of the person viewing the rotated image stack.
  You can verify the result in the small preview window in the tab. The scheme below illustrates the rotation direction of each image level in the case of positive values for Z, Y and X. In the case of negative values, the rotation direction is exactly the other way around:



- To make settings for the rotation animation, click on the Animation check box. The corresponding fields are enabled.
- In the Rotation field, select the animation type: Full turn or 360°, half turn or 180°, quarter turn or 90°, or any other angle.

- In the Frames field, enter the number of projection images to be created for the animation. The number of projection images and the animation type specify the intervals at which the projection images are created. With a half turn (angle of 180°) and a number of images of, for example, 10, an image is created in steps of 20° (not 18°), as there are only 9 steps for 10 recorded images.
  Note
  There is another calculation in the case of the full turn (angle of 360°). To prevent the first and last projection image from being recorded under the same angle (360° = 0°), the function calculates one recorded image more, but without creating the corresponding projection image. In the case of 10 images, for example, the interval between the projection images is 36° (not 40°), as 11 recorded images (i.e. 10 steps) are used in the calculation. However, only 10 projection images are created.
- In the Rotation Axis field, vary the inclination of the rotation axis. Check the changes in the preview window.
- In the Depth Factor field, enter a value to scale the z-axis of the projection images.
- Click on the Apply button to start the calculation of the projection, to display the result image in the Viewer window and to create it as a new file in the current experiment.
- To start the rotation animation, click on the Movie button.
  see Starting and Ending a Film

Creating Red-Green Stereo Views
On the **Stereo View Tab** you can create red-green stereo images, so-called anaglyphic images. Anaglyphic images create a stereoscopic sense of depth if they are viewed with corresponding 3D glasses (red-green stereo glasses).

The following settings must be made to create the two stereo images:

- In the Stereo View Options field click on the On check box to activate the function.
- In the Eye Angle field determine the angle under which the two stereo images are recorded. For example, if you enter the value 30, a stereo image is recorded at an angle of 15° each left and right from the projection axis that was set on the Rotation tab.
- Stereo images can only be created from one detection channel. If you have an image series that was recorded in several detection channels, select the desired image data set in the Detection Channel field.
- Click on the Show button to obtain a preview of the stereo images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the stereo images as a new data set. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.
- To display both stereo images in one image in the Viewer window, the buttons Channel 1, Channel 2, Single and Overlay must be pressed.
  see Viewing Detection Channel 1
  see Viewing Detection Channel 2

see Viewing a Single Image
see Viewing an Overlay Image

Additional information
The stereoscopic effect of the anaglyphic image is possible since human beings use both eyes for viewing. Since the eyes are approximately 6.5 cm (2.56 inch) apart, the brain receives two images from different perspectives. Objects located at different distances are observed at different angles. This angular difference is a measure of distance for the human brain. The difference between the similar images generated by the two eyes is processed by the brain into one image with spatial depth impression. Holding a slightly different image in front of each eye can also simulate these image differences.

In anaglyphic images, the complete stereoscopic image information is located on one image. The information of the left and right image is coded through colors. For this reason, glasses with a different color filter for each eye are needed in viewing these images. This filter separates the image information again for each eye. The red filter only passes red light, while completely is absorbing the green light. On the other hand, the green filter absorbs the red light. Each eye perceives one image; both are merged in the brain into an overall spatial image.

Icon Orthogonal Projection	Creating projection images along the orthogonal axis Creating Projection Images from Different Detection Channels

Creating a Projection Image Along the Orthogonal Axis (z-axis with horizontal xy-sections and y-axis with vertical xz-sections)

- In the Type field, select one of the three projection types: maximum projection, average projection, and transparent projection.
  see Principles and Types of Projections
- The Direction field determines which single images are used for the projection.
  Select [z] for stereoscopic series, [t] for time series or [l] for wavelength series to create the projection from all images of a detection channel, such as from image 1 to image 10 in detection channel 1.
  Select [ch] to create the projection from a certain image in all detection channels, for example from image 1 in detection channel 1 and image 1 in detection channel 2.
- Click on Rescale in the Options field to standardize the intensity values of the image to the maximum possible intensity values when creating the projection image. This ensures that the calculated gray values of the projection image remain within the value range of, for example, 0 to 255 at 8 bits. This allows for brightening dark images.
- If you click on Invert in the Options field, the projection axis is mirrored. The calculation of the projection image is thus in the opposing direction. This function makes sense above all in the case of inverse microscopes to match the calculation of the projection image to the inverse beam path of laser light.
- The Transparent Factor field is only active if the transparent projection was selected. The slider is used to vary the transparent factor a. The higher it is set, the more the intensity values from the lower levels in the image stack affect the projection image.
  see Principles and Types of Projections
- Move the slider in the Threshold field to define a threshold value. The intensity values below this value are not taken into account for the creation

of the projection image.

- Click on the Show button to obtain a preview of the projection images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the projection as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Note

> *The three projection types can also be enabled directly using the corresponding keys (Fix O. Proj / Fix Max / Fix Avg / Fix Trans). If you activate the function using the button, the projection image will just be a temporary display on the monitor. You can undo this using the Original key.*

see Projection of an Image Stack With Invariable Projection Axis
see Maximum Projection of an Image Stack With Invariable Projection Axis
see Average Projection of an Image Stack With Invariable Projection Axis
see Transparent Projection of an Image Stack With Invariable Projection Axis

SFP Icon (Simulated Fluorescence Process)	Creating SFP Projection Images Running SFP Projection Images as 3D Rotation Animation Creating a Red-Green Stereo View of the SFP Projection

Creating an SFP Projection Image

see Principles and Types of Projections

- In the **SFP** tab in the Light Direction field, you define the projection angle for the first computing step of the SFP projection. This angle symbolizes the angle of incidence of the laser beam onto the specimen. Enter one coordinate each for x and y.
- Click on Rescale in the Options field to standardize the intensity values of the image to the maximum possible intensity values when creating the projection image. This ensures that the calculated gray values of the projection image remain within the value range of, for example, 0 to 255 at 8 bits. This allows for brightening dark images.
- If you click on Invert in the Options field, the projection axis is mirrored. The calculation of the projection image is thus in the opposing direction. This function makes sense above all in the case of inverse microscopes to match the calculation of the projection image to the inverse beam path of laser light.
- If you click on Filter before Rendering in the Options field, the image stack is filtered in a lowpass filter in all three spatial directions before the SFP projection image is calculated. In this way, strong noise can be removed from the recorded images.
- If you click on Calc. Shadow in the Options field, a shadow is calculated and displayed for the SFP projection image.
- Move the slider in the Absorption field to vary the absorption coefficient a.

The higher this is set, the smaller the values calculated in the projection image. If the coefficient is set as low, many of the pixels in the projection image reach the maximum value, making it increasingly difficult to distinguish between the structures.

- Move the slider in the Threshold field to define a threshold value. The intensity values below this value are not taken into account for the creation of the projection image.
- Click on the Show button to obtain a preview of the projection images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the projection as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

In the Rotation and Stereo View tabs, you can create a rotation animation or a stereo view of the SFP projection image.

see Running Projection Images as a 3-dimensional Rotation Animation
see Creating a Red-Green Stereo View

Filter (Linear) Icon Applying low-pass and high-pass filters to images

Filters are used to enhance the quality of an image. As a rule, this means removing undesirable pixels from the image. The filter function available here a low-pass filter; is used to suppress static noise in an image that could be generated by the detector. The principle of a filter consists of offsetting the value of each pixel in an image with the values of its neighboring pixel. The filter kernel describes the number and weighting of the adjacent pixels that come to bear in the calculation of the new pixel. The kernel used here can be described using Pascal's triangle:

Filter kernel size	Pascal triangle	Scaling
	1 1	1/2
Kernel Size 3	1 2 1	1/4
	1 3 3 1	1/8
Kernel Size 5	1 4 6 4 1	1/16
	1 5 10 10 5 1	1/32
Kernel Size 7	1 6 15 20 15 6 1	1/64
	1 7 21 35 35 21 7 1	1/128
Kernel Size 9	1 8 28 56 70 56 28 8 1	1/256
	...	...

If, for example, kernel size 5 is selected, 5 pixels of the original image are used in calculating 1 pixel in the filtered image. It is always the middle pixel in the filter kernel that is the pixel to be calculated. In this case, the gray values of the 5 pixels corresponding to the weighting scale 1-4-6-4-1, are multiplied by the factors 1/16, 4/16, 6/16, 4/16, 1/16 and together added to the final value. With respect to the calculation of the pixel C in the following schematic representation this means that the gray values of pixels A, B and D, E are included in the calculation of the filtered pixel C'. C' is calculated as follows: 16x1/16 + 4x4/16 + 8x6/16 + 32x4/16 + 8x1/16 = 1 + 1 + 3 + 8 + 0,5 = 13,5

	A	B	C	D	E
Gray Values of Pixels A-E in an Original Image	16	4	8	32	8
Factors of kernel size 5	1/16	4/16	6/16	4/16	1/16

Gray value of C' in the filtered image 13,5

A calculation of this kind is performed for each pixel in the original image. At the edge of the image, the filter mask protrudes beyond the image. For these pixels, the value 0 is set.

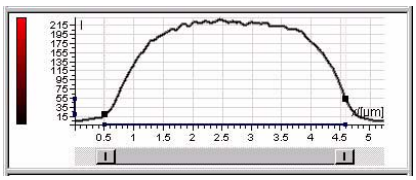
- In the Kernel Size field, select a value for the kernel size.

In the Filter Directions field, set the dimensions of the filter. Select the spatial axes to which the filter is to be applied sequentially. One-dimensional filters are applied, that is, the filter is calculated only via one axis at any given time.

Intensity profile of an unfiltered image

- In the Filter Type field, select the Smooth filter.
The low-pass filter described above is applied to the image.

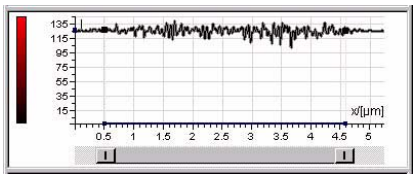
This filter type filters the high frequencies out of the image. That is, the very distinctive transitions from low to high intensity values are reduced.



Intensity profile of the image after applying the Smooth filter

This filter can be used to remove noise from an image.

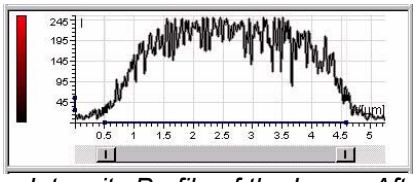
- In the Filter Type field, select the Highpass filter.
After the low-pass filter has been applied to the image, the values of the original image are subtracted from the values of the low-pass-filtered image. The result corresponds to an image that was processed with a high-pass filter.



Intensity Profile of the Image After Application of the Highpass Filter

This filter type exclusively represents the clearly defined transitions from low to high intensity values in the image.

- In the Filter Type field select the Sharp filter.
After the low-pass filter has been applied to the image, the values of the high-pass-filtered image are added to the values of the original image.



Intensity Profile of the Image After Application of the Sharp Filter

This filter type filters the low frequencies out of the image. That is, the very distinctive transitions from low to high intensity values are intensified.

This filter is also used to amplify the noise in an image.

Use the slider in the Sharp Factor field to vary the effect of the Sharp filter. If this factor is set to 0, the filter is not active.

- The value specified in the Kernel Size field as Cut-off Wavelength, is the size of the filter measured in pixels. This value is calculated on the basis of the scan parameters (enlargement of the objective, wavelength of the excitation light, scan format, electronic zoom factor...) used for each image. The value specified in the Filter Directions field as Cut-off Wavelength, is the size of the filter measured in nanometers. For example, with a value of 160 nm all wavelengths lesser than or equal to 160 nm are filtered.
- Click on the Show button to obtain a preview of the filtered images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the filtered images as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Image Tool Dialog Window, Basic Button

Schematic Structure of the Dialog Window
The numerous functions of the Image Tool dialog window are arranged on a number of levels. The gray buttons 3D, Basic, Amplitude, Materials and Multicolor on the left side of the dialog window represent the first level of the hierarchy. Each of these buttons has a group of icons that appear below the selected button. The icons are the next level on which functions are grouped. Each icon opens different tabs and fields that are displayed on the right side in the dialog window and arrange the functions on the lowest level.

The following elements can be found in each tab:

- In the Source Image drop-down list at the top of the dialog window, select the image data record to be processed.
- If you click on the Use Selected Image check box, the image data record currently displayed in the Viewer window is used for the calculation.
- If you click on the Show button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the preview window in the dialog window.
- If you click on the Apply button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the Viewer window and created as a new file in the current experiment.
- Click on the button with the arrow symbols to show the preview window on the right edge of the dialog window or to hide it. The projections triggered using Show are displayed temporarily in this preview window.
- The Close button closes the dialog window.

Leveling icon	Adapting an interpolation function to a data set to correct trend curves (e.g., skewed recording of a data set)

Procedure
This procedure aims at bringing an intensity curve that features a trend curve into a horizontal position. For this purpose, a suitable interpolation function (polynomial) is first adapted to the intensity curve. Next, the distance of the optimal interpolation function to a horizontal intensity plateau is calculated. The occurring intensity differences are added pixel by pixel to the original data set. This compensates the trend curve.

see Adaptation Function for Correcting Trend Curves

The target function to be optimized in determining the coefficients of the adaptation function is:

$$\int \| I - f(\Omega) \| d\Omega = \min$$

Procedure for calculating the adaptation function

- Select an image from the Source Image drop-down list. The drop-down list contains all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- In the Fitting Interpolation Function field select the character of the adaptation function:

Fitting Interpolation Function	Formula
Linear	$f(x) = a + bx + cy$
Bilinear	$f(x) = a + bx + cx + dxy$
Quadratic	$f(x) = a + bx + cx + dxy + fx^2$
Cubic	$f(x) = a + bx + cx + dxy + fx^2 + gxy^2 + hx^3 + iy^3$

- Select the step size in the Sample Step Width field. The step size sets the number of support positions for the polynomial. The smaller the step size, the more time-consuming and precise the calculation of the adaptation function.
- If the data set should show noise, it is recommended to hide the low intensity noise signals in the Threshold field by setting a threshold. All intensity values whose values are below the threshold are not used in the calculation. This prevents noise signals from being erroneously used as support positions for the adaptation function.
- Click on the Show button to obtain a preview of the arithmetical operations. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the calculation operation as a new data set. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Merging icon	Combining several images or data sets to a new image or data set

Voxel tab: Adaptation of different image resolutions and additional settings for calculating the resulting image

Field	Description of Function
Amplitude Image 1/2: Min	The minimum intensity value is shown here. For raw data, this value is «0». For scaled data, the actual minimum value is shown here. The minimum is adjustable so that differently scaled data sets can be adapted to each other. If a data set with a small interval of intensity values is combined with a data set featuring a large intensity value range, the first one is shown darker than the second one. Adapting the minimum values of both data sets scales them in such a way that equal parts of the intensity values of both data sets are included in the merged image.
Amplitude Image 1/2: Max	The maximum intensity value is shown here. For raw data, this value is «255» with 8-bit data or «4095» with 12-bit data. For scaled data, the actual maximum value is shown here. The maximum is adjustable so that differently scaled data sets can be adapted to each other. If a data set with a small interval of intensity values is combined with a data set featuring a large intensity value range, the first one is shown darker than the second one. Adapting the maximum values of both data sets scales them in such a way that equal parts of the intensity values of both data sets are included in the merged image.
Result: 8 bit / 12 bit	This setting defines the resolution of the merged image. An 8-bit resolution can display 256 different intensity levels. A 12-bit resolution can display 4096 different intensity levels.
Maximum Value	This option takes the brightest pixel each from the two images to be merged to calculate the corresponding pixel of the merged image.
Average Value	This option calculates the arithmetic mean from the two images to be merged to calculate the corresponding pixel of the merged image.

Note

If two strongly different images are calculated to a merged image by using the average method, the black image background causes a reduction of the image intensity!

Tab x, y, z, t, la: Definition of the stereoscopic arrangement of the output images in the resulting image before combining

Field	Description of Function
Image 1/2: Shift	The position in x/y/z (t/λ) of the output image within the merged image is set here. This value is typically «0». A value of > 0 is useful for example, if a reduction of one output image is to be shown at a certain position of the other output image. This function is also useful for lateral image series, such as those found in biomapping. see Example for Shifting an Image
Append check box	The second data set is now placed next to the first data set. The direction of the placement depends on whether the check box on the x, y or z tab was selected. Selecting this function in all the different tabs for x, y and z shifts the second output image either in only one dimension or in several dimensions.
Result: Element number	Here you can indicate which pixel represents the end of the merged image. Definition background: Absolute lengths are used to determine

	the dimensions of the merged image. If the two data sets to be merged feature the same linear extension but a different number of pixels (due to a different scan format), it must be decided whether the number of pixels or the resulting total length is to be decisive for the merged image. 📖 see Selecting a Scan Format
Result: Best Fit	The program uses the following procedure upon selecting «Best Fit»: The width of a single pixel is calculated for both images: µm/pixel. If the difference of this size between the two images is smaller than a factor of 2, this procedure uses the larger image resolution (i.e., the smaller µm/pixel value) for the resulting image. If the deviation of the image resolution between the two output images is larger than a factor of 2, the smaller image resolution (i.e., the larger µm/pixel value) is used for calculating the resulting image. The number of pixels of the resulting image (Element Number) divided by the total length results in the resolution of the resulting image. Definition background: Absolute lengths are used to determine the dimensions of the merged image. If the two data sets to be merged feature the same linear extension but a different number of pixels (due to a different scan format), it must be decided whether the number of pixels or the resulting total length is to be decisive for the merged image. 📖 see Selecting a Scan Format

Tab ch: Definition of the combination of images with different numbers of detection channels

Field	Description of Function
Image 1: Start	If two images or data sets with different numbers of detection channels are to be merged, this option can be used to define the channel number in the merged image at which the channels of the output image 1 should be inserted. 📖 see Example for merging images with different channel numbers
Image 2: Start	If two images or data sets with different numbers of detection channels are to be merged, this option can be used to define the channel number in the merged image at which the channels of the output image 2 should be inserted. 📖 see Example for merging images with different channel numbers
Check box: Append	This function distributes the two output images to different channels of the merged image.

Stereo icon	Creating projection images along the orthogonal axis Creating red-green stereo views

Creating projection images along the orthogonal axis (z-axis for horizontal xy-sections and y-axis for vertical xz-section)

- In the Type field, select one of the three projection types: maximum projection, average projection, and transparent projection.
  📖 see Principles and Types of Projections
- The Direction field determines which single images are used for the projection.
  Select [z] for stereoscopic series, [t] for time series or [l] for wavelength series to create the projection from all images of a detection channel, such

as from image 1 to image 10 in detection channel 1.
Select [ch] to create the projection from a certain image in all detection channels, for example from image 1 in detection channel 1 and image 1 in detection channel 2.

- Click on Rescale in the Options field to standardize the intensity values of the image to the maximum possible intensity values when creating the projection image. This ensures that the calculated gray values of the projection image remain within the value range of, for example, 0 to 255 at 8 bits. This allows for brightening dark images.
- If you click on Invert in the Options field, the projection axis is mirrored. The calculation of the projection image is thus in the opposing direction. This function makes sense above all in the case of inverse microscopes to match the calculation of the projection image to the inverse beam path of laser light.
- The Transparent Factor field is only active if the transparent projection was selected. The slider is used to vary the transparent factor a. The higher it is set, the more the intensity values from the lower levels in the image stack affect the projection image.
  see Principles and Types of Projections
- Move the slider in the Threshold field to define a threshold value. The intensity values below this value are not taken into account for the creation of the projection image.
- Click on the Show button to obtain a preview of the projection images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the projection as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Note

> *The three projection types can also be enabled directly using the corresponding keys (Fix O. Proj / Fix Max / Fix Avg / Fix Trans). If you activate the function using the button, the projection image will just be a temporary display on the monitor. You can undo this using the Original key.*

see Projection of an Image Stack with Invariable Projection Axis
see Maximum Projection of an Image Stack with Invariable Projection Axis
see Average Projection of an Image Stack with Invariable Projection Axis
see Transparent Projection of an Image Stack with Invariable Projection Axis

On the **Stereo View Tab** you can create red-green stereo images, so-called anaglyphic images. Anaglyphic images create a stereoscopic sense of depth if they are viewed with corresponding 3D glasses (red-green stereo glasses).

The following settings must be made to create the two stereo images:

- In the Stereo View Options field click on the On check box to activate the function.
- In the Eye Angle field determine the angle under which the two stereo images are recorded. For example, if you enter the value 30, a stereo

image is recorded at an angle of 15° each left and right from the projection axis that was set on the Rotation tab.

- Stereo images can only be created from one detection channel. If you have an image series that was recorded in several detection channels, select the desired image data set in the Detection Channel field.
- Click on the Show button to obtain a preview of the stereo images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the stereo images as a new data set. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.
- To display both stereo images in one image in the Viewer window, the buttons Channel 1, Channel 2, Single and Overlay must be pressed.
  see Viewing Detection Channel 1
  see Viewing Detection Channel 2
  see Viewing a Single Image
  see Viewing an Overlay Image

Additional information
The stereoscopic effect of the anaglyphic image is possible since human beings use both eyes for viewing. Since the eyes are approximately 6.5 cm (2.56 inch) apart, the brain receives two images from different perspectives. Objects located at different distances are observed at different angles. This angular difference is a measure of distance for the human brain. The difference between the similar images generated by the two eyes is processed by the brain into one image with spatial depth impression. Holding a slightly different image in front of each eye can also simulate these image differences.

In anaglyphic images, the complete stereoscopic image information is located on one image. The information of the left and right image is coded through colors. For this reason, glasses with a different color filter for each eye are needed in viewing these images. This filter separates the image information again for each eye. The red filter only passes red light, while completely is absorbing the green light. On the other hand, the green filter absorbs the red light. Each eye perceives one image; both are merged in the brain into an overall spatial image.

Image Separation icon	Separating single images or image segments from a data set

Here you perform the settings to separate a segment from an image or complete images from an image stack in one or several detection channels.

- Select an image from the Source Image drop-down list. The drop-down list contains all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- The dimensions x, y and z and the detection channel can freely be combined in the Dimension Selection field:

Field	**Function description**
Dimension Selection ch	The detection channels of the image or the image series are indicated here. Select the detection channels from which the image segment or the individual images are to be separated.
Dimension Selection z	The number of single images of an image series is indicated here. Enter the number of images to be separated in the Start z and End z entry fields. Use the R (Reset) button to undo the

	entries.
Dimension Selection y	The number of pixels in y that is dependent upon the scan format is indicated here. Enter the number of pixels to be separated in the Start y and End y entry fields. Use the R (Reset) button to undo the entries.
Dimension Selection x	The number of pixels in x that is dependent upon the scan format is indicated here. Enter the number of pixels to be separated in the Start x and End x entry fields. Use the R (Reset) button to undo the entries.
Step z / y / x	Here you can enter an interval within a dimension. For example, if you enter 2 in the Step z entry field, only every 2nd image is separated.

- If you want to separate all images of a detection channel from an image series, click in the ch entry field on the channel to be separated without changing the values in the entry fields of the other dimensions.
- If you want to separate complete single images from an image series, enter the corresponding numbers in the Start z and End z entry fields without changing the values in the entry fields of the other dimensions.
- If you want to separate a segment in all images of an image series, define the size of the segment in the Start x, End x and Start y, End y entry fields without changing the values in the entry fields for z.

Image Tool Dialog Window, Amplitude Button

Schematic Structure of the Dialog Window
The numerous functions of the Image Tool dialog window are arranged on a number of levels. The gray buttons 3D, Basic, Amplitude, Materials and Multicolor on the left side of the dialog window represent the first level of the hierarchy. Each of these buttons has a group of icons that appear below the selected button. The icons are the next level on which functions are grouped. Each icon opens different tabs and fields that are displayed on the right side in the dialog window and arrange the functions on the lowest level.

The following elements can be found in each tab:

- In the Source Image drop-down list at the top of the dialog window, select the image data record to be processed.
- If you click on the Use Selected Image check box, the image data record currently displayed in the Viewer window is used for the calculation.
- If you click on the Show button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the preview window in the dialog window.
- If you click on the Apply button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the Viewer window and created as a new file in the current experiment.
- Click on the button with the arrow symbols to show the preview window on the right edge of the dialog window or to hide it. The projections triggered using Show are displayed temporarily in this preview window.

- The Close button closes the dialog window.

Arithmetic With Two Images Icon	Running Arithmetical Operations with Images and/or Masks

Procedure

- Select two images from the Source Image 1 and Source Image 2 drop-down lists. The two drop-down lists contain all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- Select the desired arithmetical operation in the Operation field.

Note

A requirement for running arithmetical operations is equal dimensions or a 1/n relationship of the two data records. Equal dimensions means that two images must have the same format in x and y, and that two image stacks must have the same number of single images (z). The 1/n relationship exists when, for example, 1 single image is offset against each (nth) single image of an image stack.

The following arithmetical functions can be run with two images and/or masks:

Operation	**Result**
Addition	Adds the intensity values of the two selected images according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image\ 1}$ + Pixel Intensity $_{Source\ Image\ 2}$
Subtraction	Subtracts the intensity values of the two selected images according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image\ 1}$ - Pixel Intensity $_{Source\ Image\ 2}$
Multiplication	Multiplies the intensity values of the two selected images according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image\ 1}$ x Pixel Intensity $_{Source\ Image\ 2}$
Division	Divides the intensity values of the two selected images according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image\ 1}$ / Pixel Intensity $_{Source\ Image\ 2}$
Min	Selects for each pixel the minimum value of the two selected images according to the formula: Pixel Intensity $_{Result}$ = MIN (Pixel Intensity $_{Source\ Image\ 1}$; Pixel Intensity $_{Source\ Image\ 2}$)
Max	Selects for each pixel the maximum value of the two selected images according to the formula: Pixel Intensity $_{Result}$ = MAX (Pixel Intensity $_{Source\ Image\ 1}$; Pixel Intensity $_{Source\ Image\ 2}$)
Average	Calculates for each pixel the average of the two selected images according to the formula: Pixel Intensity $_{Result}$ = 1/2 x (Pixel Intensity $_{Source\ Image\ 1}$ + Pixel Intensity $_{Source\ Image\ 2}$)
AND	The binary representations of the pixels of the two selected

images are linked with the logical operator AND. That means that pixels in the result image are given the value 1 when the value 1 is present in both of the pixels; otherwise 0 is entered.
see Boolean Operations
You achieve meaningful results using this Boolean operator when you link an image with a binary mask. The pixel is displayed in the result image at the point where the pixels of the image meet the pixels of the mask with the value 1. The areas defined by the mask are cut out of the image.

OR — The binary representations of the pixels of the two selected images are linked with the logical operator OR. That means that pixels in the result image are given the value 1 when the value 1 is present in at least one of the two pixels; otherwise 0 is entered.
see Boolean Operations
You achieve meaningful results using this Boolean operator: 1. When you link two binary masks. The result image is a combination of the two masks. 2. When you link an image with a binary mask. The result image is an image in which the mask appears. 3. When you link two images. In contrast to the overlay image, pure intensity values are overlaid here. An RGB color addition does NOT take place here.
see Viewing an Overlay Image

XOR — The binary representations of the pixels of the two selected images are linked with the logical operator XOR. That means that pixels in the result image are given the value 1 when the two pixels have different values; otherwise 0 is entered.
see Boolean Operations
You achieve meaningful results using this Boolean operator when you create two binary masks of images and link them. The pixel is displayed in the result image at the point where the two images differ.

- In the Arithmetic Type field, select whether raw data or scale data is to be used for the arithmetical operations.
- If you have selected Raw Data, the values standardized to the interval [0..255] for 8 bits or [0..4095] for 12 bits are used for the arithmetical operations. As the calculation result can produce values extending beyond the interval, the following three methods are available to readapt them to the interval:

Raw Data Options	Result
Cut at Maximum	Values less than 0 are set to 0. All values greater than 255 for 8-bit images or 4095 for 12 bit images are set to the value 255 or 4095. This "concatenates" all the values lying outside the interval.
Absolute value	The amounts of the result data are used. This turns negative results into positive results.
Rescale	The extreme values are determined. The minimum value is assigned to the 0. The maximum value for 8-bit images is assigned to the value 255, for 12-bit images to the value 4095.

- If you have selected Scaled Data, the world coordinates, that is, the actual and non-standardized values, are used for the arithmetical operations. The standardization of the results data into the intensity interval [0..255] and/or [0..4095] is only used for the image display functions for color look-up; in contrast, the actual result values are used for the quantification functions.
- If you select 12 Bit Output in the Output Option field, each pixel in the result image is coded with 12 bits, i.e. the result values are saved in a value range from [0..4095]. In the case of scaled data, the intermediate values are interpolated.
- Click on the Show button to obtain a preview of the arithmetical operations. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the calculation operation as a new data set. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Arithmetic With Constant Icon	Running Arithmetical Operations with Images and a Constant

Procedure

- Select an image from the Source Image drop-down list. The drop-down list contains all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- Set the desired value of the constants in the Constant Value field.
- Select the desired arithmetical image operation in the Operation field.

The following arithmetical functions can be run with one image and a constant:

Operation	Result
Addition	Adds the constants pixel by pixel to the intensity values of the selected image according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image}$ + Constants
Subtraction	Subtracts the constants pixel by pixel from the intensity values of the selected image according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image}$ - Constants
Multiplication	Multiplies the constants pixel by pixel by the selected image according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image}$ x Constants
Division	Divides the selected image by the constants according to the formula: Pixel Intensity $_{Result}$ = Pixel Intensity $_{Source\ Image}$/ Constants
Min	Selects for each pixel the minimum value of the selected image and constants according to the formula: Pixel Intensity $_{Result}$ = MIN (Pixel Intensity $_{Source\ Image}$; Constants)
Max	Selects for each pixel the maximum value of the selected image and constants according to the formula: Pixel Intensity $_{Result}$ = MAX (Pixel Intensity $_{Source\ Image}$; Constants)
Average	Calculates for each pixel the average of the selected image and constants according to the formula: Pixel Intensity $_{Result}$ = 1/2 x (Pixel Intensity $_{Source\ Image}$ + Constants)
AND	The binary representations of the pixels of the constants and the image are linked with the logical operator AND. That means that pixels in the result image are given the value 1 when the value 1 is present in both of the pixels; otherwise 0 is entered. see Boolean Operations

OR	The binary representations of the pixels of the constants and the image are linked with the logical operator OR. That means that pixels in the result image are given the value 1 when the value 1 is present in at least one of the two pixels; otherwise 0 is entered. 📖 see Boolean Operations
XOR	The binary representations of the pixels of the constants and the image are linked with the logical operator XOR. That means that pixels in the result image are given the value 1 when the two pixels have different values; otherwise 0 is entered. 📖 see Boolean Operations

- In the Arithmetic Type field, select whether raw data or scale data is to be used for the arithmetical operations.
- If you have selected Raw Data, the values standardized to the interval [0..255] for 8 bits or [0..4095] for 12 bits are used for the arithmetical operations. As the calculation result can produce values extending beyond the interval, the following three methods are available to readapt them to the interval:

Raw Data Options	**Result**
Cut at Maximum	Values less than 0 are set to 0. All values greater than 255 for 8-bit images or 4095 for 12 bit images are set to the value 255 or 4095. This "concatenates" all the values lying outside the interval.
Absolute value	The amounts of the result data are used. This turns negative results into positive results.
Rescale	The extreme values are determined. The minimum value is assigned to the 0. The maximum value for 8-bit images is assigned to the value 255, for 12-bit images to the value 4095.

- If you have selected Scaled Data, the world coordinates, that is, the actual and non-standardized values, are used for the arithmetical operations. The standardization of the results data into the intensity interval [0..255] and/or [0..4095] is only used for the image display functions for color look-up; in contrast, the actual result values are used for the quantification functions.
- If you select 12 Bit Output in the Output Option field, each pixel in the result image is coded with 12 bits, i.e. the result values are saved in a value range from [0..4095]. In the case of scaled data, the intermediate values are interpolated.
- Click on the Show button to obtain a preview of the arithmetical operations. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the calculation operation as a new data set. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Bit Converter Icon	Conversion of 8-Bit Image Resolution to 12-Bit Image Resolution and Vice Versa

Procedure

- Select an image from the Source Image drop-down list. The drop-down list contains all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- In the New Resolution field, select the image resolution into which you want to convert the selected image.

- Click on the Show button to obtain a preview of the converted image. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the converted image as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Brightness & Contrast Icon	Setting image brightness and contrast

Procedure

- Select an image from the Source Image drop-down list. The drop-down list contains all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- For multichannel images, select the All Channels check box to make all changes for all channels at one time and to the same extent.
- For images that do not use the entire dynamic range, select the Linear Model check box. If the check box has been clicked, there is a linear relationship between the intensity in the output image and the result image. Whether an image uses the entire dynamic range available can be seen by creating a histogram of the image. If the histogram does not cover the entire intensity range (see Min and Max), the dynamic range is NOT being used in full.
  see Calculating a Histogram
  If you have images in which you wish to highlight the medium intensities more strongly, select the non-linear model (Linear Model check box NOT enabled).
- In the Options field, use the sliders to change the brightness and/or image contrast.

Options	**Result**
Slider Brightness	This slider adjusts the brightness of the result image. If the slider is exactly in the center, the output image and result image have exactly the same level of brightness. If the slider is moved from the center to the right, the result image becomes brighter than the output image. If the slider is moved from the center to the left, the result image becomes darker than the output image.
Slider Contrast	This slider adjusts the contrast of the result image. If the slider is exactly in the center, the output image and result image have exactly the same level of contrast. If the slider is moved from the center to the right, the result image has greater contrast than the output image. If the slider is moved from the center to the left, the result image has less contrast than the output image.

- Click on the Show button to obtain a preview of the calculation. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the calculation as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Gamma Correction Icon	Setting the Contrast Transfer Function (Gamma Curve)

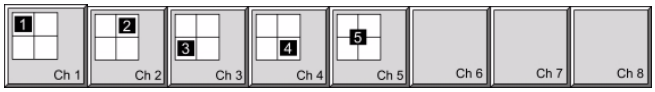
The contrast transfer function (gamma curve) can be used to influence the conversion of the output image contrast to the result image contrast in a non-linear manner.

Procedure

- Select an image from the Source Image drop-down list. The drop-down list contains all the recorded and calculated images of the experiments displayed in the Experiment Overview viewer window.
- For multichannel images, select the All Channels check box to make all changes for all channels at one time and to the same extent.
- Change the form of the contrast transfer function by adjusting the slider.
- Click on the Show button to obtain a preview of the calculation. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the calculation as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Viewing detection channels

Viewing Detection Channel 1-8



Function
Clicking the Channel 1/2/3/4/5/6/7/8 button displays the image data that has been recorded in detection channel 1-8 in the Viewer window. You can assign any of several other color look-up tables to the detection channel. This setting can be changed both in the result image and while the image is being recorded. To do so, open the Select Look-up Tables dialog window. There are two ways of opening the dialog window.

- Click the Select Look-up Tables button.
- Hold the mouse pointer over any position in the Viewer window. Click the right mouse button. Click on the LUT item in the context menu that appears.
  The color bars for the active detection channels appear in the Viewer window.
  Double-click the corresponding color bar.

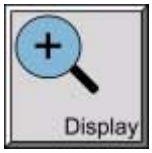
see Selecting Color Look-Up Tables (LUT)

Note

This method of assigning color look-up tables only influences the currently displayed image. As soon as you start a new scan, the color look-up tables configured in the Beam Path Setting dialog window are applied again.

see Setting the Beam Path

Zooming Image(s) in the Viewer Window



Function
Clicking the Display button opens a drop-down list that you can use to set the graphical zoom. This enlarges or reduces the image displayed in the Viewer window:

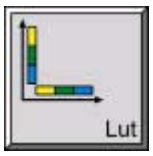
n-1	Displaying n pixel as 1 pixel in the resulting image reduces the image.
Automatic	The image is displayed in the original image format.
1-n	Displaying 1 pixel as n pixel in the resulting image enlarges the image.

Note

The Leica Confocal Software provides three different zoom functions: the graphic zoom, the 3D zoom and the electronic zoom.

see Zooming the 3D View
see Electronic Zoom

Selecting Color Maps (LUT)



Function
Use the Look-up Tables button to open a dialog window, which you can use to assign color look-up tables to the detection channels. The color look-up tables can be configured both in the result image and while the image is being recorded:

- Click the detection channel to which you want to assign a new color look-up table in the Select Channel field.
- Select the desired color look-up table in the Select LUT field.
- Click Apply to check the results in the Viewer window.

It is also possible to open the Select LUT's dialog window from the Viewer window.

- Hold the mouse pointer over any position in the Viewer window. Then push the right mouse button. Click on the LUT item in the context menu that appears.
- The color bars for the active detection channels appear in the Viewer window to the right of the image window. Double-click one of the color bars.

Loading a second color look-up table
If you hold the mouse pointer over a color bar, grab points appear at the top and bottom of the color bar. You can use these grab points to limit the current color look-up table to a specific intensity range and to load a second color look-up table:

- Drag the top grab point down or the bottom grab point up.
- Double-click the area above or below the corresponding grab point.
- The Select LUT's dialog window opens so that you can select a second color look-up table.
- The upper and lower intensity ranges are represented in the colors of the second color look-up table.

Note

This method of assigning color look-up tables only influences the currently displayed image. As soon as you start a new scan, the color look-up tables configured in the Beam Path Setting dialog window are applied again.

see Setting the Beam Path

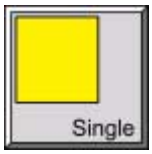
Typical Applications
Generally the selection of a suitable color look-up table for a specific use depends on the user's own judgment and needs. Experience shows however that certain color look-up tables are particularly useful for specific uses.

Color Map	Application
Green	This is commonly used for recording specimens that have been marked with FITC, Cy2, DTAF or other similar fluorochromes, which emit within the green spectral range.
Red	This is commonly used for recording specimens that have been marked with TRITC, Texas Red, Cy3, Rodamine or other similar fluorochromes, which emit within the red spectral range.
Blue	This is commonly used for recording specimens that have been marked with UV fluorochromes such as DAPI or Hoechst or other similar fluorochromes, which emit within the blue spectral range.
Gray	This is commonly used for displaying transmission recordings.
P. Color 1	This is especially suited for displaying 12 bit images.
P. Color 2, 3, 4, 5, 6	This is commonly used for recording specimens that have been marked with pH-sensitive or ion-sensitive fluorochromes and for displaying time series.
Geo (Land), Geo (Sea) Geo (Land & Sea) R & B	These are recommended for generating topographical images, i.e. for mapping surface structures. Geo Land & Sea is especially useful for visualizing depths and graduations.
Glow, Glow (Over), Glow (Under), Glow (Over & Under)	These are recommended for optimizing image contrast (the offset and gain of the detectors). Glow Over highlights in blue intensities at the upper end of the table while Glow Under represents in green intensities at the lower end of the table. Glow Over and Under is a combination of these two color tables.

Cyan, Magenta, Yellow	These are recommended for printing images. The CMY(K) colors are generally used for the printer system color output. RGB colors such as the other color look-up tables used here are applied generally for color representation on monitors. Because these two color systems vary greatly, the colors in the CMY(K) representation can differ strongly from those used in the monitor representation.

All color look-up tables are also available with inverse color flow, meaning high intensities are represented as dark and low intensities light.

Viewing the Single image



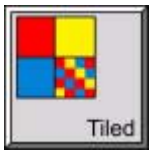
Function
Click the Single button to view only one detection channel or several detection channels in only one image in the Viewer window. The following display modes are available depending on the other options you enable simultaneously.

Button Combination	Display
Single + Channel 1 / 2 / 3 / 4 / 5	Only the selected detection channel is displayed; for an image series, the first image of the selected detection channel is displayed.
Single + Gallery + Channel 1 / 2 / 3 / 4 / 5	All individual images in an image series are displayed for the selected detection channel only.
Single + Overlay + Channel 1 / 2 / 3 / 4 / 5	A single overlay image is generated from all selected detection channels; for an image series, the first image of each selected detection channel is used.
Single + Gallery + Overlay + Channel 1 / 2 / 3 / 4 / 5	Based on the number of individual images in an image series, overlay images are generated from all selected detection channels.

Note

The Single and Tiled buttons cannot be enabled simultaneously because they carry out opposing functions.

Viewing a Multiple image



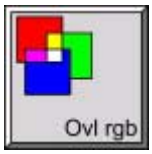
Function
If you click the Tiled button, the detection channels are displayed separately in the Viewer window. The following display modes are available depending on the other options you enable simultaneously.

Button Combination	Display
Tiled + Channel 1 / 2 / 3 / 4 / 5	All selected detection channels are displayed separately; in the case of image series, the first image in each is applied.
Tiled + Gallery + Channel 1 / 2 / 3 / 4 / 5	All individual images in an image series are displayed for all selected detection channels.
Tiled + Overlay + Channel 1 / 2 / 3 / 4 / 5	All selected detection channels are displayed both separately and, in an overlay image, together. In the case of image series, the first image in each is applied.
Tiled + Gallery + Overlay + Channel 1 / 2 / 3 / 4 / 5	All individual images of an image series are displayed both separately for all selected detection channels and, in overlay images, together.

Note

The Tiled and Single buttons cannot be enabled simultaneously because they carry out opposing functions.

Viewing an Overlay Image



Function
Clicking the Overlay button displays all selected detection channels together in an overlay image in the Viewer window. The following display modes are available depending on the other options you enable simultaneously.

Button Combination	Display
Single + Overlay + Channel 1 / 2 / 3 / 4 / 5	A single overlay image is generated from all selected detection channels; for an image series, the first image of each selected detection channel is used.
Gallery + Single + Overlay + Channel 1 / 2 / 3 / 4 / 5	Based on the number of individual images in an image series, overlay images are generated from all selected detection channels.
Tiled + Overlay +	All selected detection channels are shown

Channel 1 / 2 / 3 / 4 / 5	separately as well as together in an overlay image. In an image series, the first image is used.
Tiled + Gallery + Overlay + Channel 1 / 2 / 3 / 4 / 5	All individual images of an image series are displayed both separately for all selected detection channels and, in overlay images, together.

Use the Viewer Options dialog window to select from three different methods of color mixing for generating an overlay image:

- From the View menu, select Viewer Options.
- Click on the icon Overlay.

see Viewer Options dialog window, icon Overlay

Viewing Image Series

Viewing the First Image of a Series

Function
You can view the individual images of an image series as a film sequence. Click First to jump to the first image in the series.

Note

If the Gallery button is enabled, the First and Last, Next and Previous and Play/Stop buttons are disabled and displayed in gray. The Gallery button is used to show all of the individual images of an image series and does not allow film sequence mode.

Display the Next Image in a Series

Function
You can view the individual images of an image series as a film sequence. Click Next to jump to the next image in the series.

Note

If the Gallery button is enabled, the Next and Previous, First and Last and Play/Stop buttons are disabled and displayed in gray. The Gallery button is used to show all of the individual images of an image series and does not allow film sequence mode.

Displaying the Previous Image in a Series

Function
You can view the individual images of an image series as a film sequence. Click Previous to jump to the previous image in the series.

Note

If the Gallery button is enabled, the Next and Previous, First and Last and Play/Stop buttons are disabled and displayed in gray. The Gallery button is used to show all of the individual images of an image series and does not allow film sequence mode.

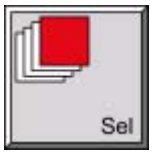
Viewing the Last Image of a Series

Function
You can view the individual images of an image series as a film sequence. Click on the Last button to jump to the last image in the series.

Note

If the Gallery button is enabled, the First and Last, Next and Previous and Play/Stop buttons are disabled and displayed in gray. The Gallery button is used to show all of the individual images of an image series and does not allow film sequence mode.

Displaying an Image From a Series



Function
When you click on the Selection button, a dialog window opens which lets you select any image of a series and display it in the Viewer window. The number of individual images for the series is displayed from 1 to n, as well as in an entry field the number of the currently displayed image.

- Use the mouse to move the slide of the scale. The corresponding image is displayed immediately.
- Enter the number of the desired image into the entry field, and click on Apply.

Starting and Ending a Film

Function
You can view the individual, recorded images of an image series as a film sequence. Use the Play/Stop button to start and stop the film. The film speed, that is the number of single images per time unit, is variable and can be set in the Viewer Options dialog window:

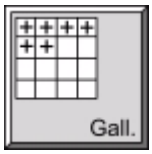
- From the View menu, select Viewer Options.
- Click the Display icon and then on the Movie tab.

see Viewer Options dialog window, Display icon

Note

If the Gallery button is enabled, the Play/Stop, First and Last, Next and Previous buttons are disabled and displayed in gray. The Gallery button is used to show all of the individual images of an image series and does not allow film sequence mode.

Viewing the Series image

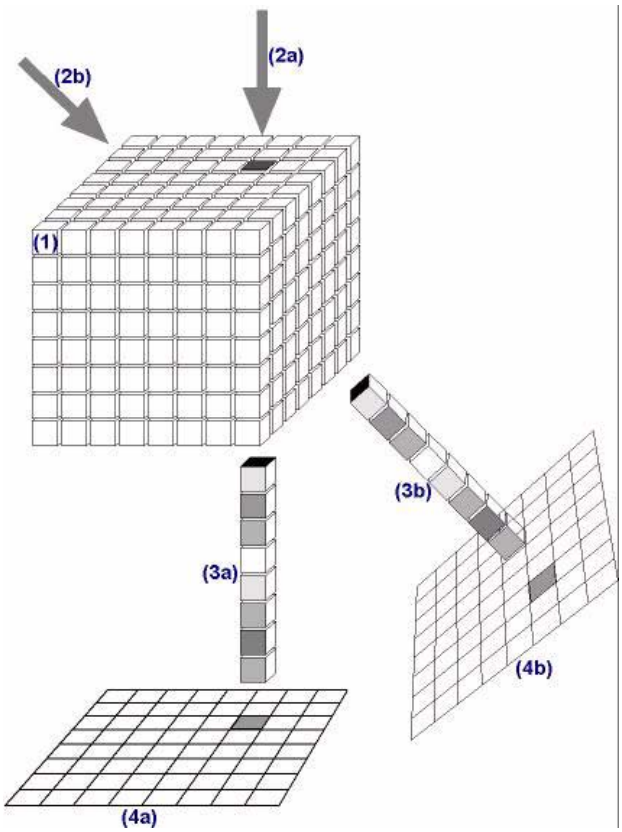


Function
If you click the Gallery button, all of the individual images in an image series are displayed in the Viewer window. The following display modes are available depending on the other options you enable simultaneously.

Button Combination	Display
Gallery + Single + Channel 1 / 2 / 3 / 4 / 5	All individual images in an image series are displayed for the selected detection channel only.
Gallery + Single + Overlay + Channel 1 / 2 / 3 / 4 / 5	Based on the number of individual images in an image series, overlay images are generated from all selected detection channels.
Gallery + Tiled + Channel 1 / 2 / 3 / 4 / 5	All individual images in an image series are displayed for all selected detection channels.
Gallery + Tiled + Overlay + Channel 1 / 2 / 3 / 4 / 5	All individual images of an image series are displayed both separately for all selected detection channels and, in overlay images, together.

Projections

Principles and Types of Projections



(2a) Projection with an invariable projection axis
(2b) Projection with a variable projection axis

Functional Principles of Projections

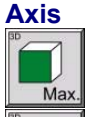
Biological specimens are recorded with a series of images. Each image is taken from a particular level in the specimen. The result is a three dimensional recording that contains the entire volume of a specimen. Each optical section (each individual image) depicts only the information from a single level in the specimen. Therefore, structures that extend throughout the entire specimen will be partially represented in several images. Projections are necessary to view these structures in their entirety. Using projection algorithms, it becomes possible to select the relevant information from many images and to depict this information in a single two-dimensional image. This method can be used to reconstruct connected structures.

Accordingly, a series of images is the foundation of any projection. The series may consist of horizontal xy-sections or vertical xz-sections. In order to best understand the functionality of a projection, it is practical to think of an image series as a stack of individual images. In turn, each individual image consists of individual laser sampling points. Furthermore, the individual sampling points or Voxels (1), (the intensity values measured in each individual image) lie on top of each other. During creation of a projection, the sampling points of the individual images, which lie on top of each other along the projection axis (2a, 2b), are scanned through all of the optical sections. Then, either the intensity value that fulfills the selection criteria or another calculated value is depicted in a two-dimensional image (4a, 4b) as a representative of all intensity values

within the column. This is repeated for each column of sampling points (3a, 3b).

Projections with invariable or variable projection axes
There are two main groups of projection types available in the Leica Confocal Software. These are distinguished by the type of projection axis. There are projections with invariable axes as well as projections with variable axes. The first type is orthogonal projections. The projection axis is always the optical axis that runs vertical to the stack of images (the z-axis with horizontal xy-cuts and the y-axis with vertical xz-cuts). In contrast, the projection axis for the second type may be freely selected. With the exception of the SFP projection, the same three projection types are available in both of the two main groups.

The Projection Types

Projection Type	Invariable Projection Axis	Variable Projection Axis
Maximum Projection		
Average Projection		
Transparent Projection		
SFP Projection	_____	

Activate the projection type either with the corresponding button or in the Image Tool dialog window. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment:

- The projections with invariable projection axes are located in the Image Tool dialog window / 3D button / Orthogonal Projection icon.
- The projections with variable projection axes are located in the Image Tool dialog window / 3D button / Projections and Animations icon.
- The SFP projection is located in the Image Tool dialog window / 3D button / SFP icon.

Maximum Projection
During maximum projection, we assume that the maximum intensity values are the relevant information for the reconstruction of a structure. For this reason, the maximum value is found in each column of sampling points and is displayed in the two dimensional projection image as representative of the entire column:

Ip = Max(Vn)
Whereby Ip is the pixel in the projection image and Vn is the investigated Voxel

Average Projection
During average projection, every intensity value with equal weighting flows into the projection image. For this reason, during average projection the arithmetic average of all intensity values is calculated in each column of sampling points. This value is displayed in the two dimensional projection image as representative of the entire column:

$$Ip = \frac{\sum_{n=0}^{N-1} Vn}{N}$$

whereby IP is the pixel in the projection image, Vn is the investigated Voxel, and N is the total number of Voxel

Transparent Projection

During transparent projection, all intensity values should be included in the projection image as well. In contrast to average projection, the intensity values from the various individual images are weighted differently. The intensity values from the lower images in the image stack are weighted less than the intensity values from the upper images. For this reason, during transparent projection the weighted mean of all intensity values is calculated in each column of sampling points. This value is displayed in the two dimensional projection image as representative of the entire column:

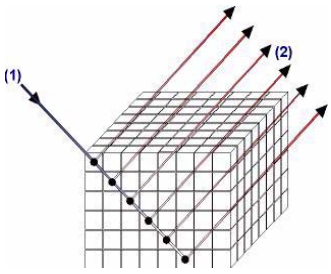
$$I_0 = V_0$$
$$I_1 = (T_n \times \alpha) \times I_0 + V_1$$
$$I_2 = (T_n \times \alpha) \times I_1 + V_2$$
$$\ldots$$
$$I_n = (T_n \times \alpha) \times I_{n-1} + V_n$$

Whereby In is the pixel in the projection image, Tn = 1 - (Vn / Vmax) is the standardization, a is the variable transparent factor, In-1 is the value of the previously calculated Voxels, and Vn is the currently investigated Voxel

Calculation of the projection image begins with the lowest and ends with the highest individual image in the image stack. Weighting of the intensity values is based on two factors.
The first factor, Tn, is calculated from the relationship of the respective intensity value to the maximum possible intensity (standardization). This factor ensures that an image's gray value range from 0 to 255 at 8 bit, for example, will not be exceeded.
The second (user adjustable) transparent factor a (0 < a < 1) determines the weighting of the previously measured intensity value. The higher the transparent factor is adjusted, the more the intensity values from the lower levels in the image stack will be included in the projection image.

SFP Projection (Simulated Fluorescence Process), mathematical simulation of a fluorescence process

This projection type uses arithmetic operations to simulate in a specimen the process of absorbing laser light and emitting fluorescent light. This procedure is common in fluorescence microscopy. It consists of two arithmetic steps.



The first step simulates the laser beam (1), which penetrates the sample

and is thereby weakened. We assume a lateral light source striking the Voxel of the image stack. The weakening of the incoming beam while traversing the image stack is represented by the following expression:

$$I_{n+1} = I_n (1 - \alpha \cdot \frac{V_n}{V_{max}})$$

Whereby In+1 is the remaining intensity of the incoming light after absorption in the nth Voxel, In is the intensity of the incoming light in the nth Voxel, a is the absorption coefficient of the laser light in the sample, Vn is the intensity in the Voxel, and Vmax is the maximum Voxel intensity. The quotient Vn / Vmax corresponds to the standardization.

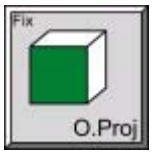
For each Voxel, the illumination strength VIn = In x Vn is calculated using the calculated intensities (In). This information is temporarily saved in a table (In = intensity of the incoming light in Voxel n, Vn = intensity of the Voxel n). This calculation is completed in one step for the entire image stack (for all Voxel in the direction of the incoming beam). The result is a table of illumination strengths.

In the second step, the established illumination strengths are used to calculate the emitted fluorescence (2). The recursive formula is used to calculate the intensity of the pixel in the projection image as representative of the Voxel in the observer's viewing direction.

$$I_{n+1} = I_n (1 - \alpha \cdot \frac{V_n}{V_{max}}) + V_{In}$$

In addition, a is the absorption coefficient of the fluorescent light in the sample and VIn is the illumination strength of the nth Voxel, calculated in the first step.

Projecting an Image Stack with an Invariable Projection Axis



Function
Using the button Fix. O. Proj., start the projection type that was used last or which was selected in the Viewer Options dialog window/ Projections icon/ Projections tab. For this function, the projection axis is always the orthogonal axis (z-axis for horizontal xy-sections, and y-axis for vertical xz-sections).

The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images—superimposed along the projection axis—are examined throughout all optical sections. From each of these columns comprised of sampling points, depending on the projection type, the maximum intensity value or the arithmetical average or, the weighted average of all intensity values, is calculated and displayed in a two-dimensional projection image representing the entire column.

see Principles and Types of Projections

- Click the Fix. O. Proj. Button. A maximum, average, or transparent projection of the current image stack is created and displayed in the Viewer window.
- You can return to the original image anytime by clicking on the Original button.

The Viewer Options dialog window allows you to indicate a threshold and to scale the intensity value range for the image.

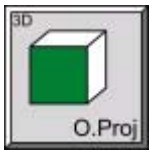
see Viewer Options Dialog Window, Projections Icon

Note

You can activate a projection using either the Fix O. Proj. Button or the Image Tool dialog window. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Projecting an Image Stack with a Variable Projection Axis (optional)



Function
The 3D Proj. Button starts the project type last used or selected in the Image Tools dialog window/ Projections and Animations icon/ Projections tab. The projection axis is freely variable.

The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis;are examined throughout all optical sections. From each of these columns comprised of sampling points, depending on the projection type, the maximum intensity value or the arithmetical average or, the weighted average of all intensity values, is calculated and displayed in a two-dimensional projection image representing the entire column.

see Principles and Types of Projections

- Click the 3D Proj. Button. A maximum, average, or transparent projection of the current image stack is created and displayed in the Viewer window.
- You can now change the projection axis by clicking in the image in the Viewer window and holding the left mouse button pressed.
- Broken support lines are displayed that encloses the symbolically

displayed image stack. Turn the image stack to the desired new position by moving the mouse pointer while holding down the left mouse button.

- Upon releasing the left mouse button, the new projection will be calculated and displayed. The projection axis is the viewing direction of the user to the newly positioned image stack.
- Use the Zoom 3D View button to enlarge or reduce the projection.
  see Zooming the 3D View
- You can return to the original image anytime by clicking on the Original button.

The Viewer Options dialog window allows you to indicate a threshold and to scale the intensity value range for the image.

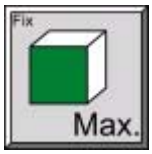
see Viewer Options Dialog Window, Projections Icon

Note

You can activate a projection by either using the 3D Proj. Button or the Image Tool dialog window. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Maximum Projection of an Image Stack with Invariable Projection Axis



Function
Using the button Fix. Max. Button starts a maximum projection. For this function, the projection axis is always the orthogonal axis (z-axis for horizontal xy-sections, and y-axis for vertical xz-sections).

The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. From each of these columns of sampling points, the maximum intensity value is displayed in the two-dimensional projection image as the representative of all intensity values within the column.

see Principles and Types of Projections

- Click the Fix. Max. Button. A maximum projection of the current image stack is created and displayed in the Viewer window.
- You can return to the original image anytime by clicking on the Original button.

In the Viewer Options dialog window, you can also adjust a threshold and scale the intensity value range present in the image.

see Viewer Options Dialog Window, Projections Icon

Note

A maximum projection can be activated either by using the Fix. Max. Button or in the Image Tool dialog window via 3D button, Orthogonal Projection icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

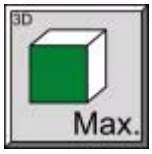
see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Note

Note the difference between a maximum projection and a topographical image, which is generated from intensity maximums. In a maximum projection, the intensity maximums are assigned color values directly. In a topographical image based on intensity maximums, the intensity maximums are assigned real z positions of the individual sampling points first and then are color-coded.

see Creating a Topographical Image

Maximum Projection of an Image Stack with Invariable Projection Axis



Function
The 3D Max. Button starts a maximum projection. The projection axis is freely variable.
The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. From each of these columns of sampling points, the maximum intensity value is displayed in the two-dimensional projection image as the representative of all intensity values within the column.

see Principles and Types of Projections

- Click on the 3D Max. Button. A maximum projection of the current image stack is created and displayed in the Viewer window.
- You can now change the projection axis by clicking in the image in the Viewer window and holding the left mouse button pressed.
- Broken support lines are displayed that encloses the symbolically displayed image stack. Turn the image stack to the desired new position by moving the mouse pointer while holding down the left mouse button.
- If you release the left mouse button, a new maximum projection is calculated and displayed. The projection axis is the viewing direction of the user to the newly positioned image stack.
- Use the Zoom 3D View button to enlarge or reduce the projection.
  see Zooming the 3D View
- You can return to the original image anytime by clicking on the Original button.

In the Viewer Options dialog window, you can also adjust a threshold and scale the intensity value range present in the image.

see Viewer Options Dialog Window, Projections Icon

Note

A maximum projection can be activated either with the 3D Max. Button or in the Image Tool dialog window with the 3D button and the Projections and Animations icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

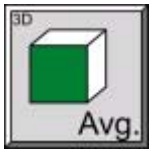
see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Note

Note the difference between a maximum projection and a topographical image, which is generated from intensity maximums. In a maximum projection, the intensity maximums are assigned color values directly. In a topographical image based on intensity maximums, the intensity maximums are assigned real z positions of the individual sampling points first and then are color-coded.

see Creating a Topographical Image

Average Projection of an Image Stack With Variable Projection Axis (optional)



Function
Use the button 3D Avg. to start an average projection. The projection axis is freely variable.
The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. From each of these columns of sampling points, the arithmetic average is calculated from all intensity values and displayed in the two-dimensional projection image as the representative of the complete column.

see Principles and Types of Projections

- Click on the 3D Avg. button to create an average projection of the current stack of images. The projection is displayed in the Viewer window.
- You can now change the projection axis by clicking in the image in the Viewer window and holding the left mouse button pressed.
- Broken support lines are displayed that encloses the symbolically displayed image stack. Turn the image stack to the desired new position by moving the mouse pointer while holding down the left mouse button.
- After you release the left mouse button, the computer calculates and displays the new average projection. The projection axis is the viewing direction of the user to the newly positioned image stack.
- Use the Zoom 3D View button to enlarge or reduce the projection.
  see Zooming the 3D View
- You can return to the original image anytime by clicking on the Original button.

In the Viewer Options dialog window, you can also adjust a threshold and scale the intensity value range present in the image.

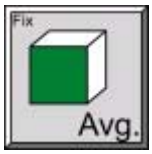
see Viewer Options Dialog Window, Projections Icon

Note

You can activate an average projection with the 3D Avg. button or in the Image Tool dialog window, 3D button/ Projections and Animations icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Average Value Projection of an Image Stack with Invariable Projection Axis



Function
Using the button Fix. Avg. button starts an average projection. For this function, the projection axis is always the orthogonal axis (z-axis for horizontal xy-sections, and y-axis for vertical xz-sections).

The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. From each of these columns of sampling points, the arithmetic average is calculated from all intensity values and displayed in the two-dimensional projection image as the representative of the complete column.

see Principles and Types of Projections

- Click the Fix. Avg. button. An average projection of the current image stack is created and displayed in the Viewer window.
- You can return to the original image anytime by clicking on the Original button.

In the Viewer Options dialog window, you can also adjust a threshold and scale the intensity value range present in the image.

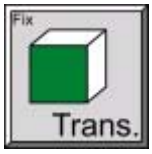
see Viewer Options Dialog Window, Projections Icon

Note

An average projection can be activated either by using the Fix. Avg. button or in the Image Tool dialog window via 3D button, Orthogonal Projection icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Transparent Projection of an Image Stack with Invariable Projection Axis



Function
Using the button Fix. Trans., start the transparent projection. For this function, the projection axis is always the orthogonal axis (z-axis for horizontal xy-sections, and y-axis for vertical xz-sections).

The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. From each of these sampling point columns, a weighted average is calculated from all intensity values, which is then displayed in the two-dimensional projection image as a representative of the entire column.

see Principles and Types of Projections

The user can vary the weighting of the sampling points to calculate the averages by setting the corresponding factors (transparent factor) in the Viewer Options dialog window. Furthermore, you can indicate a threshold and scale the intensity value range for the image.

see Viewer Options Dialog Window, Projections Icon

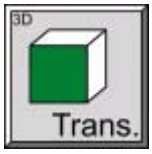
- Click the Fix. Trans. button. A transparent projection of the current image stack is created and displayed in the Viewer window.
- You can return to the original image anytime by clicking on the Original button.

Note

You can activate a transparent projection using the Fix. Trans. button. Alternately, it can be activated using the Image Tool dialog window/ 3D button/ Orthogonal Projection icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Transparent Projection of an Image Stack with Variable Projection Axis (optional)



Function
You can activate a transparent projection using the 3D Trans. The projection axis is freely variable.
The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. From each of these sampling point columns, a weighted average is calculated from all intensity values, which is then displayed in the two-dimensional projection image as a representative of the entire column.

see Principles and Types of Projections

The user can vary the weighting of the sampling points to calculate the averages by setting the corresponding factors (transparent factor) in the Viewer Options dialog window. Furthermore, you can indicate a threshold and scale the intensity value range for the image.

see Viewer Options Dialog Window, Projections Icon

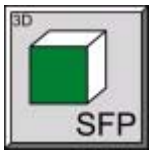
- Click the 3D Trans. button. A transparent projection of the current image stack is created and displayed in the Viewer window.
- You can now change the projection axis by clicking in the image in the Viewer window and holding the left mouse button pressed.
- Broken support lines are displayed that encloses the symbolically displayed image stack. Turn the image stack to the desired new position by moving the mouse pointer while holding down the left mouse button.
- Upon releasing the left mouse button, the new transparent projection will be calculated and displayed. The projection axis is the viewing direction of the user to the newly positioned image stack.
- Use the Zoom 3D View button to enlarge or reduce the projection.
  see Zooming the 3D View
- You can return to the original image anytime by clicking on the Original button.

Note

A transparent projection can be activated using either the 3D Trans. button or alternately, the Image Tool dialog window/ 3D button/ Projections and Animations icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Creating SFP projections of an image stack (optional)



Function
The SFP button starts an SFP projection, the mathematical simulation of a fluorescence process.
The basis for a projection is an image stack, i.e. a series of horizontal xy-sections or vertical xz-sections. When a projection is generated, the sampling points of the individual images superimposed along the projection axis are examined throughout all optical sections. In the first step, the laser beam is simulated penetrating the sample and diminishing. In the second step, using the determined light density, the simulated fluorescence for each Voxel is calculated.

see Principles and Types of Projections

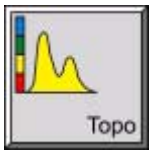
- Click the SFP button. A SFP projection of the current image stack is created and displayed in the Viewer window.
- You can now change the projection axis by clicking in the image in the Viewer window and holding the left mouse button pressed.
- Broken support lines are displayed that encloses the symbolically displayed image stack. Turn the image stack to the desired new position by moving the mouse pointer while holding down the left mouse button.
- Upon releasing the left mouse button, the new SFP projection will be calculated and displayed. The projection axis is the viewing direction of the user to the newly positioned image stack.
- You can return to the original image anytime by clicking on the Original button.

Note

This SFP projection can be activated using the SFP button and the Image Tool dialog window/ 3D button/ SFP icon. If you activate the function using the button, the projection image will just be a temporary display on the monitor. When activated through the dialog window, the projection image will be stored as a new file in the current experiment.

see Image Tool Dialog Window, 3D Button
see Creating an Experiment

Creating a Topographical image



Function
Use the Topography function to select specific intensity data from an image stack, i.e. a series of xy or xz-sections, and transfer them into a two-dimensional topographical image. This function examines the sampling points (voxels) that are superimposed along the z-axis throughout all optical sections. From each of these columns of sampling points, only the intensity value that is met by the selection criterion is displayed in the two-dimensional topographical image as the representative of all values within the column.

You can make your selection based on either the maximum intensity or the center of mass of the measured intensities. If you want to represent the intensity maximums in the topographical image, only the sampling point at which the maximum intensity was measured is selected. When determining the center of mass, a mean value is calculated from all superimposed sampling points (the center of mass of the area that is limited by the curve of the measured intensity values).

The intensity maximum or center of mass is then assigned the real z position of the corresponding sampling point and then color-coded. Based on this assignment, a topographical image **maps the real surface structure** of the specimen. Using the default settings, higher structures appear light and deeper structures dark.

Use the Viewer Options dialog window to set the selection criterion for the topographical image:

- From the View menu, select Viewer Options.
- Click on the Surface Calculation icon to view the Topography tab.

see Viewer Options dialog window, Surface Calculation icon

By applying a second step, you can display the topographical image as a three-dimensional graphical image using the 3D View function.

see Creating the 3D View

Typical Applications
Displaying the image data in a topographical image is especially informative in material scientific research. For the application of quantification functions, the topography function is indispensable.

Viewing the Original image

Function
Click the Original button to undo a projection image or topographical image. The Viewer window then displays the recorded raw data of the image again.

Creating 3D Views

Creating the 3D View

Function
Use the 3D View button to display a two-dimensional data record three-dimensionally. When viewing an image series, the data record of the series that is currently being viewed in the Viewer window is always the one applied. You can display a single xy-section or xz-section from the raw data or a result image, such as a topographical image or projection image, in the 3D view. Depending on the dimension represented or calculated in the output image, either intensity values or height values are mapped to the z-axis of the 3D view when the 3D view is generated.

Note

The spatial representation of intensity in a 3D view leads easily to the assumption that the topography of the specimen is being represented. Note however that you can only display ***the real surface structure*** *of a specimen if you have generated a topographical image first.*

see Creating a Topographical Image

The 3D view can be generated in three different modes: surface, wireframe or isolines. Set the display mode in the Viewer Options dialog window:

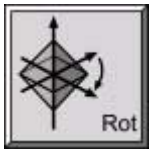
- From the View menu, select Viewer Options.
- Click the Surface View icon and then click the Visualization tab.

see Viewer Options Dialog Window, Surface View Icon

Note

A separate 3D view is created for each detection channel. Therefore it is not possible to generate a single 3D view from one overlay image (Overlay button).

Rotating the 3D View



Function
Use the Rotate button to rotate a 3D view in all three spatial directions. Note that the 3D view is rotated around a fixed point, which is located in the center of the image.

- Hold the mouse pointer over any position in the 3D view.
- Press and hold the left mouse button.
- Move the mouse pointer in the direction in which you want to rotate the 3D view.

Note

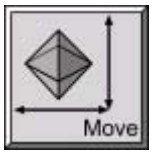
While the 3D view is being turned, the resolution of the image is decreased in order to speed up processing. When the left mouse button is released, the image is displayed with its original resolution.

As an alternative to rotating a 3D view manually, the Viewer Options dialog window also provides the option of specifying the angles of rotation for the three spatial axes:

- From the View menu, select Viewer Options.
- Click the 3D icon and then click the Navigation tab.
- You can then specify the angles of rotation for all three axes in the Rotation field.

see Viewer Options Dialog Window, 3D Icon

Moving the 3D View



Function
Use the Move button to shift a 3D view:

- Hold the mouse pointer over any position in the 3D view.
- Press and hold the left mouse button.
- Move the mouse pointer in the direction in which you want to move the 3D view.

Note

While the 3D view is being moved, the resolution of the image is decreased in order to speed up processing. When the left mouse button is released, the image is displayed with its original resolution.

As an alternative to moving a 3D view manually, the Viewer Options dialog window also provides the option of specifying the coordinate values for the position of the image:

- From the View menu, select Viewer Options.
- Click the 3D icon and then click the Navigation tab.
- Modify the coordinate values for the x-axis and z-axis in the Translation field.

see Viewer Options Dialog Window, 3D Icon

Zooming the 3D View

Function
Use the Zoom button to magnify and shrink a 3D view steplessly while maintaining the aspect ratio. This function only adjusts the scale of, i.e. zooms, the generated image. It cannot be used to improve its resolution.

To zoom in on the 3D view:	Click anywhere inside the image window of the Viewer window and, while pressing the left mouse button, drag the mouse pointer **toward the lower edge**.
To zoom out of the 3D view:	Click anywhere inside the image window of the Viewer window and, while pressing the left mouse button, drag the mouse pointer **toward the upper edge**.

Note

While the 3D view is being zoomed, the resolution of the image is decreased in order to speed up processing. When the left mouse button is released, the image is displayed with its original resolution.

As an alternative to zooming a 3D view manually, the Viewer Options dialog window also allows you to change the zoom scale by specifying numerical values:

- From the View menu, select Viewer Options.
- Click the 3D icon.
- You can then vary the zoom factor in the Navigation tab by changing the y value in the Translation field.

see Viewer Options Dialog Window, 3D Icon

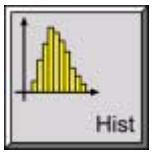
Note

The Leica Confocal Software provides three different zoom functions: 3D zoom, electronic zoom and graphical zoom.

see Electronic Zoom
see Viewer Options Dialog Window, Display Icon

Measuring and Analysis Functions

Calculating a Histogram



Function
The Histogram function measures the frequency of a measurement variable, displays it in a curve and in addition, calculates various statistical values. The histogram is measured from the data set selected in the Viewer window. This data set can be a spatial series, a time series or a wavelength series. The statistical values are calculated dependent on the size of the third dimension; height (z) for spatial series, time (t) for time series, wavelength (λ) for wavelength series.

- Click on the Histogram button.
- A viewer window opens that displays the measurement curve for each detection channel and the statistical values:

Parameter	Meaning	Formula
# Pixel	The total number of pixels that are included in the calculation of the histogram. This corresponds to the configured scan format.	

Mean	The arithmetical mean value	$\mu(I) = \frac{1}{N_{Pixel}} \sum_i I_i$
Maximum	Maximum value	Max (I)
Minimum	Minimum value	Min (I)
Variance	Variance	$VAR(I) = \frac{1}{N-1} \sum_i (I_i - \mu(I))^2$
Average Deviation	Average deviation	$DISP(I) = \frac{1}{N-1} \sum_i \lvert I_i - \mu(I) \rvert$
Standard Deviation	Standard deviation	$s(I) = \sqrt{VAR(I)} = \sqrt{\frac{1}{N-1} \sum_i (I_i - \mu(I))^2}$
Mean Energy	Average image energy	$I^2_{mean} = \frac{1}{N_{Pixel}} \sum_i I_i^2$
Root Mean Square (RMS)	Root mean square value	$RMS = \sqrt{\frac{1}{N_{Pixel}} \sum_i I_i^2}$
Skewness	Skewness of the distribution	$Skew(I) = \frac{1}{N} \sum_i \left[\frac{I_i - \mu(I)}{\sqrt{VAR(I)}} \right]^3$

Some of the parameters are specified in scientific exponential notation, e.g. Pixel = 3.28e+005 = 327680 (corresponds to a scan format of 640 x 512).

Note

Using the maximum and minimum values of intensity, the gain values and offset values can be optimally configured for the detectors.

Measuring a Profile Within a Region of Interest (ROI)

Function
The Profile (z) function measures a measurement variable within a region of interest, displays it in a curve and calculates various statistical values. The profile is measured from the data record selected in the Viewer window. This data set can be a spatial series, a time series or a wavelength series. The statistical values are calculated dependent on the size of the third dimension; height (z) for spatial series, time (t) for time series, wavelength (λ) for wavelength series.

- Click on the Profile (z) button and draw a region of interest (ROI) in the image. The buttons used to define a region of interest are activated together with the Profile (z) button.
  📖 see Defining the Region of Interest (ROI) as Ellipse
  📖 see Defining the Region of Interest (ROI) as a Polygon

see Defining the Region of Interest (ROI) as a Rectangle
see Automatically Defining the Region of Interest (ROI)
see Selecting and Shifting the Region of Interest (ROI)
see Deleting the Region of Interest (ROI)

- A window opens automatically to display the measurement curve for every detection channel as well as the statistical values.

Parameter	Meaning	Formula
# pixel (ROI)	Number of pixels within the selected region of interest	
Area	Area of the region of interest	$A = \int_0^A d_A\, A$
Length	Height of image stack (spatial series) Recording time (time series) Bandwidth (wavelength series)	$L = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$
Mean Amplitude	The arithmetical mean	$\mu(I) = \frac{1}{N_{Pixel}} \sum_{Pixel} I_i$
Maximum Amplitude	Maximum value	Max (I)
Maximum amplitude position	Position of maximum value	Arg Max I (z), (t), (λ)
Minimum Amplitude	Minimum value	Min (I)
Minimum amplitude position	Position of minimum value	Arg Min I (z), (t), (λ)
Average Deviation	Average deviation	$DISP(I) = \frac{1}{N-1} \sum_i \lvert I_i - \mu(I) \rvert$
Standard Deviation	Standard deviation	$s(I) = \sqrt{VAR(I)} = \sqrt{\frac{1}{N-1} \sum_i (I_i - \mu(I))^2}$
Variance	Variance	$VAR(I) = \frac{1}{N-1} \sum_i (I_i - \mu(I))^2$

The following four measurement values appear only when the Two-Point Measurement function is selected in the Viewer Options dialog window.

- Select the View menu, the Experiment Overview option, and the Charts icon.

see Viewer Options Dialog Window, Charts Icon

- In the Measurement field you can switch two-point measurement on or off by selecting 2 Point or Off.

I(µm); I(s); I(nm)	Intensity measured at measuring point 1 (z, t, λ)	$I(z_1)$; $I(t_1)$; $I(l_1)$
I(z); I(t); I(λ)	Intensity measured at measuring point 2 (z, t, λ)	$I(z_2)$; $I(t_2)$; $I(l_2)$
dI, dz	Difference of values between measuring point 1 and 2	$\lvert I(z_1) - I(z_2) \rvert$; $\lvert I(t_1) - I(t_2) \rvert$, $\lvert I(l_1) - I(l_2) \rvert$

d(z), (t), (λ)	Height (z), recording time (t), bandwidth (λ) between the measuring points 1 and 2	$\|z_1 - z_2\|$; $\|t_1 - t_2\|$, $\|l_1 - l_2\|$

Typical Applications
This function can be used to determine the extreme values of the intensity within an image stack. They in turn allow for the optimized setting of the detectors. In addition, this function can be used to determine the emission maximum of a wavelength series. By adapting the width and the position of the screens in front of the detectors, more emission light from the specimen can be recorded than with conventional optical filters. In time series, this function can be used to determine the time of the highest fluorescence activity.

Note

This quantification function is only available off-line (only available for previously recorded data records).

Measuring a Profile Along a Line Segment

Function
The Profile function measures a measurement variable along a line segment, displays it in a curve and calculates various statistical values. The profile is measured from the data record selected in the Viewer window. This data set can be a spatial series, a time series or a wavelength series. The statistical values are calculated dependent on the size of the third dimension; height (z) for spatial series, time (t) for time series, wavelength (λ) for wavelength series.

- Click on the Profile button. The measurement section is displayed as a white line in the image in the Viewer window.
  You can change the length and position of the line segment by clicking the line and, while clicking and holding the grab point with the left mouse button, dragging it to the desired position.
- A window opens automatically to display the measurement curve for every detection channel as well as the statistical values.

Parameter	Meaning	Formula
Length	The length of the measured section	$L = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$
Mean Amplitude	Arithmetic mean of measurement variable	$\mu(I) = \frac{1}{N_{Pixel}} \sum_{Pixel} I_i$
Maximum Amplitude	Maximum value of measurement variable	Max (I)
Minimum Amplitude	Minimum value of measurement variable	Min (I)

Average Deviation	Mean standard deviation of the values from the average value of the measurement variable	$DISP(I) = \frac{1}{N-1}\sum_i \lvert I_i - \mu(I)\rvert$
Standard Deviation	Standard deviation of the values of the measurement variable	$s(I) = \sqrt{VAR(I)} = \sqrt{\frac{1}{N-1}\sum_i (I_i - \mu(I))^2}$
Variance	Variance of the measurement variable	$VAR(I) = \frac{1}{N-1}\sum_i (I_i - \mu(I))^2$

The following four measurement values appear only when the Two-Point Measurement function is selected in the Viewer Options dialog window.

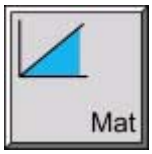
- Select the View menu, the Experiment Overview option, and the Charts icon.
  see Viewer Options Dialog Window, Charts Icon
- In the Measurement field you can switch two-point measurement on or off by selecting 2 Point or Off.

Parameter	Meaning	Formula
I(µm); I(s); I(nm)	Value measured at measuring point 1 (z, t, λ)	$I(z_1)$; $I(t_1)$; $I(l_1)$
I(µm); I(s); I(nm)	Value measured at measuring point 2 (z, t, λ)	$I(z_2)$; $I(t_2)$; $I(l_2)$
dI, dz	Difference of values between measuring point 1 and 2	$\lvert I(z_1) - I(z_2)\rvert$; $\lvert I(t_1) - I(t_2)\rvert$, $\lvert I(l_1) - I(l_2)\rvert$
d(z), (t), (λ)	Height (z), recording time (t), bandwidth (λ) between the measuring points 1 and 2	$\lvert z_1 - z_2\rvert$; $\lvert t_1 - t_2\rvert$, $\lvert l_1 - l_2\rvert$

Note

This quantification function is only available off-line (only available for previously recorded data records).

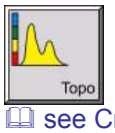
Measuring Surfaces and Volumes



Function
The Materials function calculates surfaces and volumes of the three-dimensional, spatial data set.
Click on the Materials button to automatically open a window in which a measurement curve and the size of the accumulated volume are displayed depending on the z position. Accumulated volume means that for each z position the value of the volume of the data set lying below it is calculated.

A requirement for calculating the surfaces and volumes is a typographical

image. Thus, the Materials button is only active if the Topography button was used before to create a topographical image of the data set. Only one detection channel can be quantified at a time.



see Creating a Topographical Image

Parameter	Meaning
Scanned Area A	This value represents the horizontal projection of the surface of a xy section.
Surface Area A'	This value represents the actual surface of a xy section. In relation to the horizontal projection the actual surface is always at least as large, in general even larger.
Ratio A'/A	This value represents the relation of surface to base area. The larger it is, the more the two-dimensional surface is embedded in the three-dimensional space.

The following four measurement values appear only when the Two-Point Measurement function is selected in the Viewer Options dialog window.

- Select the View menu, the Experiment Overview option, and the Charts icon.
  see Viewer Options Dialog Window, Charts Icon
- In the Measurement field you can switch two-point measurement on or off by selecting 2 Point or Off.

V(z<=upper position value)	This value represents the volume located below the upper position value of the measurement slider (right corner point of the slider).
V(z<=lower position value)	This value represents the volume located below the lower position value of the measurement slider (right corner point of the slider).
dV	Volume between the position values of the slider
dz	Distance between the position values of the slider

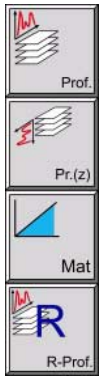
Note

This quantification function is only available off-line (only available for previously recorded data records).

Copying Quantification Graphs to the Annotation Sheet

Function
The Snap Quantification Data function copies the data calculated by the quantification functions to the annotation sheet. The calculated values for the following quantification functions, which are also displaying an evaluation graph, can be copied to an annotation sheet:

see Creating an Annotation Sheet



see Measuring a Profile Along a Line Segment

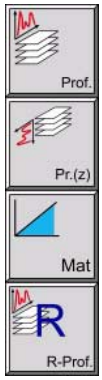
see Measuring a Profile Within a Region of Interest (ROI)

see Measuring Surfaces and Volumes

see Measuring Roughness Along a Line Segment

Printing Quantification Graphs

Function
The Print Quantification Data function sends the data calculated by the quantification functions to the standard printer. The calculated values for the following quantification functions, which are also displaying an evaluation graph, can be printed:



see Measuring a Profile Along a Line Segment

see Measuring a Profile Within a Region of Interest (ROI)

see Measuring Surfaces and Volumes

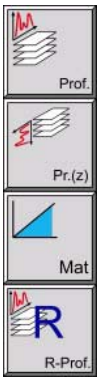
see Measuring Roughness Along a Line Segment

This function is also available in the context menu within the quantification function window:

- Move the mouse pointer into the view window opened by a quantification function.
- Click the right mouse button, and select the Print menu item.

Exporting Quantification Data

Function
Using he Export Quantification Data function, you can export the data calculated by the quantification functions as ASCII standard data. This enables you to read and edit data in Excel. The calculated values for the following quantification functions, which are also displaying an evaluation graph, can be exported:



see Measuring a Profile Along a Line Segment

see Measuring a Profile Within a Region of Interest (ROI)

see Measuring Surfaces and Volumes

see Measuring Roughness Along a Line Segment

This function is also available in the context menu within the quantification function window:

- Move the mouse pointer into the view window opened by a quantification function.
- Click the right mouse button, and select the Export menu item.

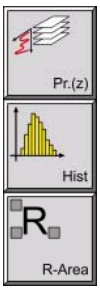
Defining the Region of Interest (ROI) as an Ellipse

Function
Using the Ellipse button, you can define an elliptic Region of Interest (ROI) within the image:

- Click the Ellipse button.
- Click the left mouse button on the image position where you would like to place a ROI corner.
- Then, keeping the left mouse button pressed, drag the mouse pointer diametrically to the opposite corner to define the second corner of the ROI.

see Selecting and Moving the Region of Interest (ROI)
see Moving and Rotating a Region of Interest (ROI)
see Deleting Regions of Interest (ROIs)

Other Requirements
The definition of a region of interest in the image is required for quantification functions. Therefore, the Ellipse button is deactivated until you activate a quantification function using one of the following buttons:



see Measuring a Profile Within a Region of Interest (ROI)
see Calculating a Histogram
see Measuring Roughness Within a Region of Interest (ROI)

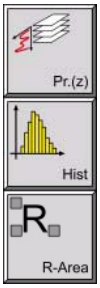
Defining the Region of Interest (ROI) as a Polygon

Function
Using the Polygon button, you can define any polygonal Region of Interest (ROI) within the image:

- Click the Polygon button.
- Click the left mouse button on the image position where you would like to place a ROI corner.
- In order to define the other polygon corners, release the mouse button, move the cursor to the next polygon corner, and click the left mouse button again. Repeat this step for each desired polygon corner.
- If you want to draw the polygon by hand, keep the left mouse button pressed and draw the ROI into the image.
- Close the polygon with a double click.

see Selecting and Moving the Region of Interest (ROI)
see Moving and Rotating a Region of Interest (ROI)
see Deleting Regions of Interest (ROIs)

Other Requirements
The definition of a region of interest in the image is required for quantification functions. Therefore, the Polygon button is deactivated until you activate a quantification function using one of the following buttons:

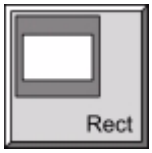


see Measuring a Profile Within a Region of Interest (ROI)

see Calculating a Histogram

see Measuring Roughness Within a Region of Interest (ROI)

Defining the Region of Interest (ROI) as a Rectangle



Function

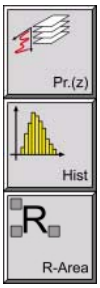
Using the Rectangle button, you can define a rectangular Region of Interest (ROI) within the image:

- Click the Rectangle button.
- Click the left mouse button on the image position where you would like to place a ROI corner.
- Then, keeping the left mouse button pressed, drag the mouse pointer diametrically to the opposite corner to define the second corner of the ROI.

see Selecting and Moving the Region of Interest (ROI)
see Moving and Rotating a Region of Interest (ROI)
see Deleting Regions of Interest (ROIs)

Other Requirements

The definition of a region of interest in the image is required for quantification functions. Therefore, the Rectangle is deactivated until you activate a quantification function using one of the following buttons:



see Measuring a Profile Within a Region of Interest (ROI)

see Calculating a Histogram

see Measuring Roughness Within a Region of Interest (ROI)

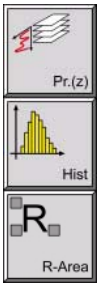
Automatically Defining the Region of Interest (ROI)

Function
You can use the Wizard button to have the computer define any structure in the image as a Region of Interest (ROI) automatically in the image. Depending on which of the buttons you have clicked, an elliptical, rectangular or polygonal region of interest is placed around the structure.

- Click on the Ellipse, Rectangle or Polygon buttons then on the Wizard button.
- Double-click with the left mouse button on the structure in the image that you want to mark as region of interest.
- The structure is automatically marked as an elliptical, rectangular or polygonal region of interest.

see Defining the Region of Interest (ROI) as Ellipse
see Defining the Region of Interest (ROI) as a Polygon
see Defining the Region of Interest (ROI) as a Rectangle
see Deleting Regions of Interest (ROIs)

Other Requirements
The definition of a region of interest in the image is required for quantification functions. The Wizard button is only enabled when you have previously enabled a quantification function using one of the following buttons:



see Measuring a Profile Within a Region of Interest (ROI)
see Calculating a Histogram
see Measuring Roughness Within a Region of Interest (ROI)

Selecting and Moving the Region of Interest (ROI)

Function
Using the Select ROI button, you can select and move a Region of Interest (ROI) within the image:

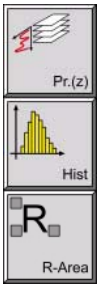
- Click first the Select ROI button and then the ROI you want to move.
- Move the mouse pointer into the ROI.
- As soon as the mouse pointer changes to a crossed double arrow, press and hold the left mouse button.
- Keeping the left mouse button pressed, move the ROI.

You can change the size of the region of interest you have drawn:

- Hold the mouse pointer over one of the ROI's edges until a double arrow appears. Press the left mouse button to shift the shape of the ROI in one direction.
- If you place the mouse pointer exactly over a corner of the ROI, you can change its shape in two directions at once.

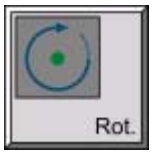
see Moving and Rotating a Region of Interest (ROI)
see Deleting a Region of Interest (ROI)

Other Requirements
The definition of a region of interest in the image is required for quantification functions. Therefore, the Select ROI is deactivated until you activate a quantification function using one of the following buttons:



see Measuring a Profile Within a Region of Interest (ROI)
see Calculating a Histogram
see Measuring Roughness Within a Region of Interest (ROI)

Moving and Rotating the Region of Interest (ROI)



Function
Using the Rotate ROI button, you can move and rotate a Region of Interest (ROI) within the image:

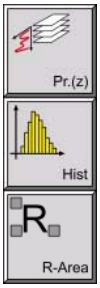
- Click first the Rotate ROI button and then the region of interest you want to rotate.
- Place the mouse pointer exactly on the corner of the selected region of interest.
- As soon as the mouse pointer changes to a double arrow, press and hold the left mouse button.
- Keeping the left mouse button pressed, rotate the region of interest. The region of interest will be rotated around its center point.

You can change the size of the region of interest you have drawn:

- Hold the mouse pointer over one of the ROI's edges until a double arrow appears. Press the left mouse button to shift the shape of the ROI in one direction.

see Moving and Rotating a Region of Interest (ROI)
see Deleting Regions of Interest (ROIs)

Other Requirements
The definition of a region of interest in the image is required for quantification functions. Therefore, the Rotate ROI is deactivated until you activate a quantification function using one of the following buttons:

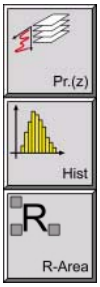


see Measuring a Profile Within a Region of Interest (ROI)
see Calculating a Histogram
see Measuring Roughness Within a Region of Interest (ROI)

Deleting Regions of Interest (ROIs)

Function
The Clear button deletes all defined ROIs at once. To delete an individual ROI, click the Select ROI button, then the ROI in the image, and press the DELETE key on the keyboard.

Other Requirements
The definition of a region of interest in the image is required for quantification functions. Therefore, the Clear is deactivated until you activate a quantification function using one of the following buttons:



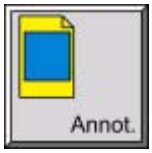
see Measuring a Profile Within a Region of Interest (ROI)

see Calculating a Histogram

see Measuring Roughness Within a Region of Interest (ROI)

Documenting Data

Creating an Annotation Sheet



Function
Use the Annotation button to open a window in which you can prepare recorded images for presentation purposes. If you have opened the annotation sheet, the Snap, Line, Rectangle and Text buttons are also enabled. Use these buttons to copy the image loaded in the Viewer window into the annotation sheet, to highlight specific areas of the copied image with lines and rectangles and to enter an image comment into a text field.

see Copying an Image
see Drawing a Line
see Drawing a Rectangle
see Adding a Text Field

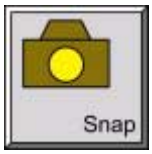
If you hold the mouse pointer over the annotation sheet and press the right mouse button, a context menu containing the following commands appears:

Command	Function
Line	Use this to insert a line of default size onto the annotation sheet.
Rectangle	Use this to insert a rectangle of default size onto the annotation sheet.
Text	Use this to insert a text field with a default font onto the annotation sheet.
Zoom	Use this to select from four magnifications for viewing the annotation sheet.
Grid	This displays a grid on the annotation sheet, which cannot be printed.

The Line, Rectangle and Text commands can be activated both from the context menu and by clicking their corresponding buttons.

Saved annotation sheets are given the *.ano file extension.

Copying an Image into the Annotation Sheet



Function
If you click the Snap button, the image loaded in the Viewer window is copied onto the annotation sheet. If you mark the copied image and then press the right mouse button, a context menu containing the following commands appears:

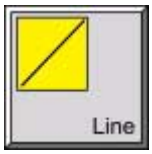
Command	Function
Original size	This displays the image in its original size.
Fit to page	This enlarges the image to the size of the annotation sheet.
Bring to front	This brings the image to the foreground.
Send to back	This sends the image to the background.
Delete	This deletes the image.

You can change the size of the image by dragging one of the image grab points. To move the image without changing its size, mark the image and move it while pressing the left mouse button.

Note

The Snap button can be applied only if an annotation sheet has been opened using the Annotation button first and the Viewer window has been clicked.

Drawing a Line on the Annotation Sheet



Function
Use the Line button to draw a line on the annotation sheet. On the annotation sheet, click the position where you want the line to begin. Keeping the left mouse button depressed, drag the mouse pointer across the page to the position where you want the line to end. If you mark the line and then press the right mouse button, a context menu containing the following commands appears:

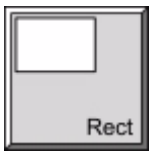
Command	Function
Style	This opens a dialog window where you can configure the style, thickness and length of the line.
Color	This opens a dialog window for selecting a color for the line.
Bring to front	This brings the line to the foreground.
Send to back	This sends the line to the background.
Delete	This deletes the line.

You can change the length of the line by dragging one of its grab points. To move the line without changing its size, click the center grab point and move the line while pressing and holding the left mouse button.

Note

The Line button can be applied only if the annotation sheet has been opened using the Annotation button first.

Drawing a Rectangle on the Annotation Sheet



Function
Use the Rectangle button to draw a rectangle on the annotation sheet. On the annotation sheet, click the position where you want the corner of the rectangle to be. Keeping the left mouse button depressed, drag the mouse pointer across the page to define the size of the rectangle. If you mark the rectangle and then press the right mouse button, a context menu containing the following commands appears:

Command	Function
Style	This opens a dialog window where you can configure, among other options, the style and thickness of the line.
Color	This opens a dialog window for selecting a color for the rectangle.

Bring to front	This brings the rectangle to the foreground.
Sent to back	This sends the rectangle to the background.
Delete	This deletes the rectangle.

You can change the size of the rectangle by dragging one of the image grab points of the rectangle. To move the rectangle without changing its size, click in the middle of the rectangle and move it while pressing and holding the left mouse button.

Note

The Rectangle button can be applied only if the annotation sheet has been opened using the Annotation button first.

Adding a Text Field to an Annotation Sheet

Function
Use the Text button to insert a text field onto the annotation sheet. On the annotation sheet, click the position where you want the text field to be placed. Keeping the left mouse button depressed, drag the mouse pointer across the page to define the size of the text field. If you mark the text field and then press the right mouse button, a context menu containing the following commands appears:

Command	Function
Font	This opens a dialog window used to select from various fonts and other formatting options.
Transparent	This makes the background visible through the text field.
Bring to front	This brings the text to the foreground.
Send to back	This sends the text to the background.
Delete	This deletes the text.

You can change the size of the text field by dragging one of the image grab points of the text field. To move the text field without changing its size, click in the middle of the text field and move it while pressing and holding the left mouse button.

Note

The Text button can be applied only if the annotation sheet has been opened using the Annotation button first.

Printing

Function
You can trigger the print command either by clicking the Print button or from the Printer Selection dialog window. To open this dialog window, select the Print option in the File menu. The image loaded in the Viewer window or an annotation sheet can be printed.

When you initiate the Print command, the image currently loaded in the Viewer window or the currently open annotation sheet is printed using the default printer and default page layout. You can change these default settings in the Printer Selection dialog window to suit your needs. Use the following buttons provided in the dialog window to do so:

Button	Function
Printer setup	This opens the dialog window for selecting a printer and changing the printer settings.
Print	This prints the image or the annotation sheet.
Background color	Use this to open a dialog window for selecting a background color for the page.
Center on page	This centers the image on the page.
Fit to page	This option changes the size of the image to fit the size of the printable area.
Aspect ratio	The height-to-width ratio remains the same when the size of the image changes.

Use the Image field to change the height (Size Y) and width (Size X) of the image and to define a top margin (Offset Y) and a left margin (Offset X). The image size and margins cannot be modified if the Fit to page option is enabled. If you have enabled Center on Page, you can change the size but not the margins.

Check the results of your settings in the Print Preview field. Use the Page field to specify the height and width of the printable area (which is not identical to the paper size). The two bottom lines of the dialog window display the currently installed printer and configured paper size.

Note

Close the Printer Selection dialog window by clicking OK to save the new settings for the active Viewer window. This allows you to store different printer settings and page layouts for different images. However, these settings are not permanent and are lost when you exit the Leica Confocal Software.

Data Handling

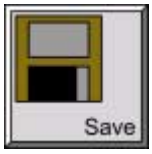
Opening a File

Function
With the Leica Confocal Software, you can open various file types using the Open button

File Type	Description
Experiment (*.lei)	A Leica-specific, binary data format. When the experiment is loaded, both the image data and the experiment settings are loaded.
Annotation sheet (*.ano)	A Leica-specific, binary data format. The elements on the annotation sheet, such as images, texts and graphic images, are each available as individual objects.
Tiff files (*.tif)	These are Leica image files in single and multi Tiff format. External files in RGB-Tiff format can be loaded as well.

see Creating an Experiment
see Creating an Annotation Sheet

Saving a File



Function
Click the Save button to save the data of the current experiment (*.lei) or the current annotation sheet (*.ano).

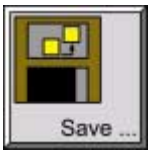
If you use the Save button to save an experiment or annotation sheet for the first time, the Save As dialog window opens.

Note

It is easy to inadvertently overwrite your original data using the Save function. To avoid doing so, use the Save As function to save an experiment that has already been saved once or more under a new name.

see Saving a File As...

Saving a File as...



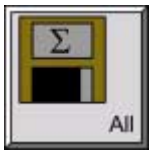
Function
Use the Save As button to save an experiment (*.lei) or annotation sheet (*.ano) while giving it a specific name and file type.

When saving an experiment, a folder is created at the file level with the name of the experiment. This folder then contains the description file (*.lei) for the experiment as well as the individual image files. The format of the description file is Leica-specific and binary. This file contains the parameter settings and the color information (in the form of color look-up tables) for each image belonging to the experiment.

The image files of an experiment can be saved in *tif* or *raw* format. The standard format, *tif*, also contains the experiment settings and the color information for the images. In the *raw* format, just the image data is saved.

Typical Applications
The advantage of the *raw* format is the smaller file size, but this is only significant for image recordings with relatively small amounts of data. For example, if you record an image series with a large number of individual images but at a low scan format, you can save and open it faster in *raw* format than in *tif*.

Storing All Data



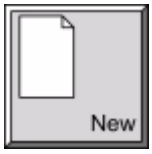
Function
Use the Save All button to save experiments (*.lei) or annotation sheets (*.ano) while giving them a specific name and file type.

When saving an experiment, a folder is created at the file level with the name of the experiment. This folder then contains the description file (*.lei) for the experiment as well as the individual image files. The format of the description file is Leica-specific and binary. This file contains the parameter settings and the color information (in the form of color look-up tables) for each image belonging to the experiment.

The image files of an experiment can be saved in *tif* or *raw* format. The standard format, *tif*, also contains the experiment settings and the color information for the images. In the *raw* format, just the image data is saved.

Typical Applications
The advantage of the *raw* format is the smaller file size, but this is only significant for image recordings with relatively small amounts of data. For example, if you record an image series with a large number of individual images but at a low scan format, you can save and open it faster in *raw* format than in *tif*.

Creating an Experiment



Function
Click the New Experiment button to open a new Viewer window, which creates a new experiment. An experiment is a file that consists of one ore more individual images or image series. This allows you to keep several images, each recorded using different scan parameters, or edited versions of images in one experiment. The format of the experiment file (*.lei) is Leica-specific.

User-specific Adaptation

Saving the Viewer Window as a Template

Function
Using the Template button, you can save a user-defined design of the Viewer window as a template. The elements of the Viewer window that can be shown or hidden, are the buttons the color bars of the color look-up tables and the Experiment legend.

see Viewer Window

Clicking the Template button opens a dialog window that you can use to enter a name for the template. This configuration of the Viewer window is saved and will be loaded each time you open a file or create a new experiment.

Additional information
In the Tools menu, click on Options and select the Viewer Template tab to access the user-defined templates. In the Leica Templates list box, you can choose from the following predefined templates:

Viewer Template	Design
Viewer (Pure)	The view window consists only of the image window.
Viewer (LUT)	The view window consists of the image window and the color bars of the color look-up tables.

Viewer (Standard)	The view window consists of the buttons, the image window, the color bars of the color look-up table, and the Experiment legend.

In the Personal Templates List box, the user-defined templates are entered. Using the Add Active Viewer and Remove Template buttons, you can save a new template or delete an existing one.

In the Tools menu, click on Options to indicate in the Workspace tab how many view windows, if any, Viewer should open when the Leica Confocal Software is started.

Controlling Functions Using the Control Panel

Function
At frequent points during an image recording, various parameters have to be reset. For this reason, the control panel can be used to control functions quickly and directly. You can freely allocate the radio buttons of the control panel to a selection of functions:

- In the View menu, select the Status Bars option and then Control Panel Status Bar.

The status bar for the control panel is displayed at the bottom of the user interface of the Leica Confocal Software. Viewed from left to right, the status bar consists of the icon for the control panel, the seven fields that represent the radio buttons of the control panel, and three buttons.

- Click on one of the fields arranged in the same way as the radio buttons of the control panel.
- A list is opened where you can select the desired function. The name of the allocated function is shown in the field.

If you have assigned a function to all the radio buttons, you can save this configuration as a template.

- Click on the left of the three buttons located on the right side of the status bar.
- A dialog window is opened in which you can give the configuration a name and save it.
- Click on the middle button to select and load a configuration.

In addition to these user-defined configurations, you can also access other configurations predefined at the factory:

- Click on the right of the three buttons located on the right edge of the status bar.
- This opens a dialog window listing all the configurations for the control panel.

The configurations set as default, indicated by an **L** (Leica), can only be loaded but not modified. The user-defined configurations are created in the drop-down list under User and marked with a **U** (User). Click one of the configurations and then press the right mouse button to open a context menu, which contains the following commands:

Command	Function
Set as default template	The configuration is loaded as the default configuration when the software is started.
Remove default setting	The setting of this configuration as default setting is undone.
Load	Loads the configuration.
Rename (only for **U**)	Use this to change the name of the configuration.
Delete (only for **U**)	The configuration is deleted.

Optional Software packages

Materials

Image Tool dialog window, Materials button (optional)

Schematic Structure of the Dialog Window
The numerous functions of the Image Tool dialog window are arranged on a number of levels. The gray buttons 3D, Basic, Amplitude, Materials and Multicolor on the left side of the dialog window represent the first level of the hierarchy. Each of these buttons has a group of icons that appear below the selected button. The icons are the next level on which functions are grouped. Each icon opens different tabs and fields that are displayed on the right side in the dialog window and arrange the functions on the lowest level.

The following elements can be found in each tab:

- In the Source Image drop-down list at the top of the dialog window, select the image data record to be processed.
- If you click on the Use Selected Image check box, the image data record currently displayed in the Viewer window is used for the calculation.
- If you click on the Show button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the preview window in the dialog window.
- If you click on the Apply button at the bottom of the dialog window, the selected calculation is activated and the result image is displayed in the Viewer window and created as a new file in the current experiment.
- Click on the button with the arrow symbols to show the preview window on the right edge of the dialog window or to hide it. The projections triggered using Show are displayed temporarily in this preview window.
- The Close button closes the dialog window.

Filter (Linear) Icon Applying low-pass and high-pass filters to images

Filters are used to enhance the quality of an image. As a rule, this means removing undesirable pixels from the image. The filter function available here, a low-pass filter is used to suppress static noise in an image that could be generated by the detector. The principle of a filter consists of

taking the value of each pixel in an image and setting it off against the values of the adjacent pixels. The filter kernel describes the number and weighting of the adjacent pixels that come to bear in the calculation of the new pixel. The kernel used here can be described using Pascal's triangle:

Filter kernel size	Pascal triangle	Scaling
	1 1	1/2
Kernel Size 3	1 2 1	1/4
	1 3 3 1	1/8
Kernel Size 5	1 4 6 4 1	1/16
	1 5 10 10 5 1	1/32
Kernel Size 7	1 6 15 20 15 6 1	1/64
	1 7 21 35 35 21 7 1	1/128
Kernel Size 9	1 8 28 56 70 56 28 8 1	1/256
...	...	...

If, for example, kernel size 5 is selected, 5 pixels of the original image are used in calculating 1 pixel in the filtered image. It is always the middle pixel in the filter kernel that is the pixel to be calculated. In this case, the gray values of the 5 pixels corresponding to the weighting scale 1-4-6-4-1, are multiplied by the factors 1/16, 4/16, 6/16, 4/16, 1/16 and together added to the final value. With respect to the calculation of the pixel C in the following schematic representation this means that the gray values of pixels A, B and D, E are included in the calculation of the filtered pixel C'. C' is calculated as follows: 16x1/16 + 4x4/16 + 8x6/16 + 32x4/16 + 8x1/16 = 1 + 1 + 3 + 8 + 0,5 = 13,5

	A	B	C	D	E
Gray values of pixels A-E in the original image	16	4	8	32	8
Factors of kernel size 5	1/16	4/16	6/16	4/16	1/16
Gray value of C' in the filtered image			13,5		

A calculation of this kind is performed for each pixel in the original image. At the edge of the image, the filter mask protrudes beyond the image. For these pixels, the value 0 is set.

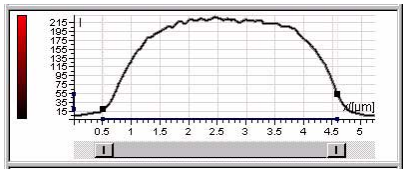
- In the Kernel Size field, select a value for the kernel size.

  In the Filter Directions field, set the dimensions of the filter. Select the spatial axes to which the filter is to be applied sequentially. One-dimensional filters are applied, that is, the filter is calculated only via one axis at any given time.

Intensity profile of an unfiltered image

- In the Filter Type field, select the Smooth filter.
  The low-pass filter described above is applied to the image.

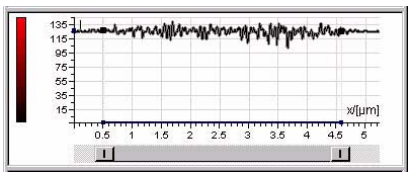
  This filter type filters the high frequencies out of the image. That is, the very distinctive transitions from low to high intensity values are reduced.



Intensity profile of the image after applying the Smooth filter

This filter can be used to remove noise from an image.

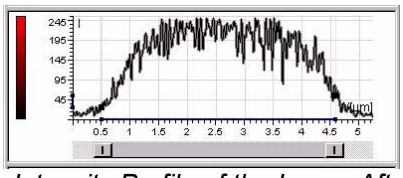
- In the Filter Type field, select the Highpass filter.
  After the low-pass filter has been applied to the image, the values of the original image are subtracted from the values of the low-pass-filtered image. The result corresponds to an image that was processed with a high-pass filter.



Intensity Profile of the Image After Application of the Highpass Filter

This filter type displays only the very distinctive transitions from low to high intensity values in the image.

- In the Filter Type field select the Sharp filter.
  After the low-pass filter has been applied to the image, the values of the high-pass-filtered image are added to the values of the original image.



Intensity Profile of the Image After Application of the Sharp Filter

This filter type filters the low frequencies out of the image. That is, the very distinctive transitions from low to high intensity values are intensified.

This filter is also used to amplify the noise in an image.

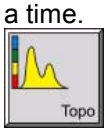
Use the slider in the Sharp Factor field to vary the effect of the Sharp filter. If this factor is set to 0, the filter is not active.

- The value specified in the Kernel Size field as Cut-off Wavelength, is the size of the filter measured in pixels. This value is calculated on the basis of the scan parameters (enlargement of the objective, wavelength of the excitation light, scan format, electronic zoom factor...) used for each image. The value specified in the Filter Directions field as Cut-off Wavelength, is the size of the filter measured in nanometers. For example, with a value of 160 nm all wavelengths lesser than or equal to 160 nm are filtered.
- Click on the Show button to obtain a preview of the filtered images. Show starts the calculation and displays the resulting images in the Image Tool dialog window.
  Or
  Click on the Apply button to create the filtered images as a new data record. Apply starts the calculation, displays the resulting images in the Viewer window and saves them as a new file in the current experiment.

Measuring Roughness Along a Line Segment

Function
The Roughness Profile function measures height, distance, and angular values of a surface along a specific line and displays these values in a curve. The height profile is measured from the data record selected in the Viewer window. This data record should be a spatial series.

A topographical image is the prerequisite for the generation of a height profile. For this reason, the Roughness Profile button is active only when a topographic image of the data record has been created in advance with the Topography button. Only one detection channel can be quantified at a time.



see Creating a Topographical Image

- Click on the Roughness Profile button. The measurement section is displayed as a white line in the image in the Viewer window.
  You can change the length and position of the line segment by clicking the line and, while clicking and holding the grab point with the left mouse button, dragging it to the desired position.
- A window opens automatically to display the measurement curve for every detection channel as well as the statistical values.

Parameter	Meaning	Formula
Evaluation length: l(P)	Length of the plotted section, according to DIN EN ISO 4287	$L=\sqrt{(x_2-x_1)^2+(y_2-y_1)^2}$
Max. Peak: Pp	Height of the highest profile peak (based on the average height), according to DIN EN ISO 4287	Max (Pp)
Min. Valley: Pn	Depth of the greatest profile valley (based on the average height), according to DIN EN ISO 4287	Min (Pv)
Deviation: Pa	Arithmetic average of the profile ordinates within the measured section (average height) according to DIN EN ISO 4287	$P_a=\frac{1}{l}\int_0^l \lvert Z(x)\rvert dx$ with $Z(x)=Z_i-\overline{Z}$
RMS: Pq	Root mean square value of the profile ordinates within the measured section, according to DIN EN ISO 4287	$P_q=\sqrt{\frac{1}{l}\int_0^l \lvert Z^2(x)\rvert dx}$ with $Z(x)=Z_i-\overline{Z}$

The following four measurement values appear only when the Two-Point Measurement function is selected in the Viewer Options dialog window.

- Select the View menu, the Experiment Overview option, and the Charts icon.
  see Viewer Options Dialog Window, Charts Icon
- In the Measurement field you can switch two-point measurement on or off by selecting 2 Point or Off.

Parameter	Meaning	Formula
h (position of the slide's left measurement point)	Profile height at the position of the slide's left measurement point	$h_1 (l_1)$
h (position of the slide's right measurement point)	Profile height at the position of the slide's right measurement point	$h_2 (l_2)$
Step Height dh	The profile's height difference between the slide's left and right measurement points	$\lvert h_2 - h_1 \rvert$
dx	Distance between the slide's left and right corners	$\lvert l_2 - l_1 \rvert$
alpha	The angle of the (left measurement point to right measurement) connection line from horizontal	$\arctan\left(\frac{dh}{dx}\right)$

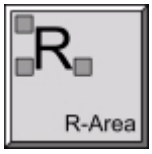
Saving the measurement values
The software can save the values on the positions of both measurement points as well as the height difference between the measurement points.

- Select the View menu, Experiment Overview option, Surface Measure icon.
  see Viewer Options Dialog Window, Surface Measure Icon
- To save the values, click on the Remember button in the field Multipoint Measurement. The Clear button deletes them again.

Note

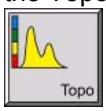
This quantification function is only available off-line (only available for previously recorded data records).

Measuring Roughness Within a Region of Interest (ROI)



Function
The Roughness Area function measures height, distance, and angular values of a surface within a region of interest. The profile is measured from the data record selected in the Viewer window. This data record should be a spatial series.

A topographical image is the prerequisite for the generation of a height profile. For this reason, the Roughness Area button is active only when a topographic image of the data record has been created in advance with the Topography button.



see Creating a Topographical Image

- Click on the button Roughness Area and draw a region of interest (ROI) into the image. The buttons with which you define a ROI are activated together with the Roughness Area button.
  see Defining the Region of Interest (ROI) as an Ellipse
  see Defining the Region of Interest (ROI) as a Polygon
  see Defining the Region of Interest (ROI) as a Rectangle
  see Automatically Defining the Region of Interest (ROI)
  see Selecting and Moving the Region of Interest (ROI)
  see Deleting Regions of Interest (ROIs)
- A window opens automatically to display the statistical values.

Parameter	Meaning	Formula
# Pixel	Number of pixels within the selected region of interest	
Area	Area	$A = \int_0^A dA\ A$
Avg. Height	Average height of the profile ordinates	$H = \frac{1}{A}\int_0^A Z(x,y)\,dA$
Pa	Arithmetic average of the profile ordinates within the measured section (average height) according to DIN EN ISO 4287	$P_a = \frac{1}{A}\int_0^A \lvert Z(x,y)\rvert\,dA$ with $Z(x,y) = Z_i - \bar{Z}$
RMS (Pq)	Root mean square value of the profile ordinates within the measured section, according to DIN EN ISO 4287	$Pq = \frac{1}{A}\int_0^A \lvert Z^2(x,y)\rvert\,dA$ with $Z(x,y) = Z_i - \bar{Z}$
Min Valley	Minimal height of the profile ordinates	Min (Pv)

(Pn)	(based on the average height)	
Max Peak (Pp)	Maximal height of the profile ordinates (based on the average height)	Max (Pp)

Note

This quantification function is only available off-line (only available for previously recorded data records).

Glossary

Aberration, chromatic	*An optical image distortion conditional on the varying refraction of light rays of different wavelengths on a lens. Thus light rays of shorter wavelengths have longer focal distances than light rays of longer wavelengths.*
Aberration, spherical	*An optical image distortion conditional on the varying distance of paraxial light rays of the same wavelength from the optic axis. Light rays that travel through outer lens zones have shorter focal distances than rays that travel through the lens center (optic axis).*
Achromatic	*Describes a correction class for objectives. The chromatic aberration for two wavelengths is corrected for objectives of this type. Usually an objective of this type is corrected to a wavelength below 500nm and above 600nm. Furthermore, the sine condition for one wavelength is met. The image curvature aberration is not corrected.*
Airy disc	*The Airy disc refers to the inner, light circle (surrounded by alternating dark and light diffraction rings) of the diffraction pattern of a point light source. The diffraction discs of two adjacent object points overlap some or completely, thus limiting the spatial resolution capacity.*
Aliasing	*An image distortion caused by a sampling frequency that is too low in relation to the signal frequency.*
AOTF Acousto-Optical Tunable Filter	*The acousto-optical tunable filter is an optic transparent crystal that can be used to infinitely vary the intensity and wavelength of radiated light. The crystal generates an internal ultrasonic wave field, the wavelength of which can be configured to any value. Radiated light is diffracted vertically to the ultrasonic wave field like through a grid.*
Aperture, numerical	*Aperture is the sine of the opening angle under which light enters into the front lens of a microscope objective; its symbol is NA. The aperture influences both the light intensity and the resolution capacity of an objective optical system. Since various media can be present between specimen and objective lens (such as the embedding medium for the specimen), the numerical aperture (NA = n * sin a) is usually applied as the unit of measurement for the light intensity and the resolution capacity.*
Apochromatic	*Describes a correction class for objectives. The chromatic aberration for three wavelengths is corrected for objectives of this type (usually 450nm, 550nm and 650nm) and the sine condition for at least two colors is met. The image curvature aberration is not corrected.*
Working distance	*The distance from the front lens of an objective to the focal point. For a variable working distance, the gap between the front lens of the objective and the cover slip or uncovered sample is specified. Usually objectives with large working distances have low numerical apertures, while high-aperture objectives have small working distances. If a high-aperture objective with a large working distance is desired, the diameter of the objective lens has to be made correspondingly large. These, however, are usually low-correction optic systems, because maintaining extreme process accuracy through a large lens diameter can only be achieved with great effort.*
Image curvature aberration	*The curved surface to which a microscopic image is to be clearly and distinctly mapped is described as image curvature aberration. It is conditional on the convex shape of the lens and makes itself apparent as an error due to the short focal distances of microscope objectives. Here the object image is not in focus both in the center and at the periphery at the same time. Objectives that are corrected for image curvature aberration are called plane objectives (plane = flat image field).*
Bleaching, optical	*The destruction of fluorochromes, so-called fluorochromes, by intense lighting. In fluorescence microscopy, fluorochromes are excited with laser light to a high state of energy, the Singlet state. When the excited molecules return to their normal energy state, a fluorescent signal is emitted. If the intensity of the*

	excitation is too high however, the color molecules can change via intercrossing from a Singlet state to a triplet state. Due to the significantly longer life of triplet states (phosphorescence), these excited molecules can react with triplet-oxide and be lost for further fluorescence excitation.
Refraction index	*The factor by which the light velocity in an optical medium is less than in a vacuum.*
Dichroic	*Dichroic filters are interference filters at an angle of incidence of light of 45°. The transmissivity or reflectivity of dichroites depends on a specific wavelength of light. For an **RSP** 510 filter (**r**eflection **s**hort **p**ass)), for example, the excitation light below 510 nm is reflected and the excitation light above this value is transmitted. The transmission values are generally between 80% and 90% and the reflection values between 90% and 95%.*
Double dichroite	*Double dichroic filters are interference filters at an angle of incidence of light of 45°. The transmissivity or reflectivity of double dichroites depends on two specific wavelengths of light. For a **d**ouble**d**ichroite DD 488/568, for example, the excitation light at 488 nm and 568 nm is reflected and the excitation light above these values is transmitted. The transmission values are generally at 80% and the reflection values are between 90% and 95%.*
Filter, digital, phase-true	*A digital filter consists of a computing rule used to modify image data. Filters are always applied to remove disturbing image components. A phase-true filter ensures that quantifiable image values do not change through filtering and remain a requirement for standardized measuring methods (e.g., characterization of surfaces in accordance with ISO).*
Fluorescence microscopy	*A light-optical contrast process for displaying fluorescent structures. Auto-fluorescent samples have a so-called primary fluorescence. They do not need to be enriched with additional, fluorescent substances. Secondary fluorescent substances, on the other hand, have to be treated with appropriate dyes or stains called fluorochromes. Specific dyeing methods additionally allow the precise localization of the stained structure elements of an object. Fluorescence microscopy allows both potential morphological examinations and the ability to carry out dynamic examinations on a molecular level.*
Fluorite objectives	*Describes a correction class for objectives. Fluorite objectives are semi-apochromatic, meaning their degree of correction lies between the achromatic and apochromatic.*
Frame	*A frame corresponds to the acquisition of a single optical section. For example, if a single optical section is acquired 4 times (to average the data and to eliminate noise), then 4 frames are created for this optical section.*
Immersion objective	*A microscopic objective, developed with the requirements for applying immersion media. The use of incorrect or no immersion medium with an immersion objective can lead to resolution loss and impairment of the correction.*
Confocality	*While the optical design of conventional microscopes allows the uniform detection of focused and unfocussed image components, the confocal principle suppresses the structures found outside of the focal plane of the microscope objective. Screens are implemented in optically conjugated locations to achieve this. They function as point light source (excitation screen) and point detector (detection screen). The diameter of the detection screen, along with the wavelength and numerical aperture of the objective being used, determines the axial extension of an optical section (optical resolution).*
Reflection short pass filter	*Reflection short pass filters are interference filters that transmit short-wave light while reflecting long-wave light. An optical reflection short pass filter is characterized by the reading of the wavelength edge at which the filter changes from transmission to reflection (50% threshold).*
Reflection long pass filter	*Reflection long pass filters are interference filters that reflect short-wave light but are transparent for long-wave light. An optical reflection long pass filter is characterized by the reading of the wavelength edge at which the filter changes from reflection to transmission (50% threshold).*
Empty	*A magnification without additional gain of information. Empty magnification is*

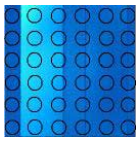
magnification	*used as soon as distances are displayed that are smaller than the optical resolution. Magnifications with a larger scale than that of the empty magnification do not provide any additional information about the object but, instead, only diminish the focus and the contrast.*
Neutral filter	*Neutral filters are semi-reflective glass plates. They are used to distribute the light path independent of wavelength. The incident light is partially reflected and partially transmitted. Neutral filters are usually placed at a 45° angle in the path of the beam. The ratings of a neutral filter **RT** 30/70, for example, **r**eflects 30% of the excitation light and **t**ransmits 70%.*
Phase visualization	*The principle of phase visualization as used by Leica is an optimized alternative method to ratiometric displaying. The main area of application lies in measuring ion concentrations in physiology. In contrast with ratiometric procedures, phase visualization obtains more information on the specimen. In addition, this method allows for adapting the display of physiological data to the dynamics of the human eye. Detailed information on the principle of phase visualization can be obtained directly from Leica Microsystems Heidelberg GmbH.*
Pixel	*An acronym based on the words, picture and element. A pixel represents the smallest, indivisible image element in a two-dimensional system. In this documentation, both the sampling points of the specimen as well as the image points are referred to as pixels.*
Plane objectives	*Describes a correction class for objectives. The image curvature aberration is corrected for objectives of this type. Correcting this error requires lenses with stronger concave surfaces and thicker middles. Three types of plane objectives, plane achromate, plane apochromate and plane fluorite, are based on the type of additional correction for chromatic aberration.*
ROI	*Abbreviation for "Region of Interest". ROI encloses an area for which a measurement analysis is to be performed. On top of that, an ROI can also designate the area of a specimen to be scanned (ROI scan).*
Signal-to-noise ratio	*The ratio of signals detected in the specimen to the unwanted signals that are caused randomly by various optic and electronic components, which are also recorded by the detector.*
Stokes shift	*The Stokes shift is a central term in fluorescence microscopy. If fluorescent molecules are excited with light of a specific wavelength, they radiate light of another, larger wavelength. This difference between excitation light and fluorescent light is referred to as Stokes shift. Without Stokes shift, separating the high-intensity excitation light from the low-intensity fluorescence signals in a fluorescence microscope would not be possible.*
Triple dichroic	*Triple dichroic filters are interference filters at an angle of incidence of light of 45°. The transmissivity or reflectivity of triple dichroites depends on three specific wavelengths of light. For a **t**riple**d**ichroite TD 488/568/647, for example, the excitation light at 488 nm, 568 nm and 633 nm is reflected and the excitation light above these values is transmitted. The transmission values are generally at 80% and the reflection values are between 90% and 95%.*
Dry objective	*A microscopic objective used without immersion media. Between the objective lens and the specimen is air.*
Voxel	*An acronym based on the words, volume and pixel. A Voxel represents the smallest, indivisible volume element in a three-dimensional system. In this documentation, both the volume elements of the specimen as well as the 3D image points are referred to as voxels.*

Appendix

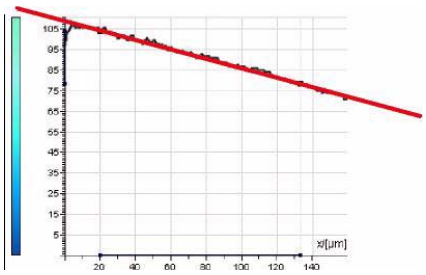
Adaptation Function For Correcting Trend Curves

see Image Tool Dialog Window, Basic Button

1. Selecting the step width, that is, the distance of the support positions

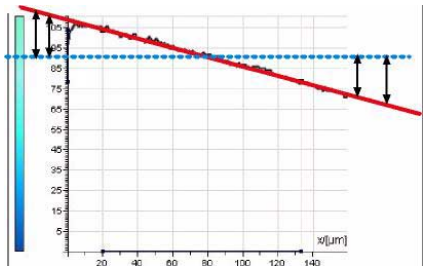


2. Selecting the character of the interpolation function to be adapted

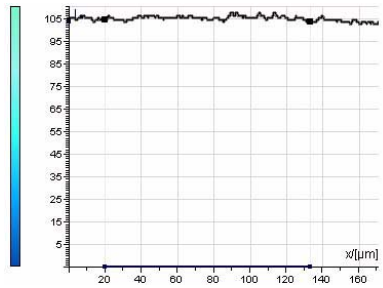


3. Calculating the interpolation function f(x) = a + bx + cy

4. Adding the intensity differences to the leveling of the trend curve

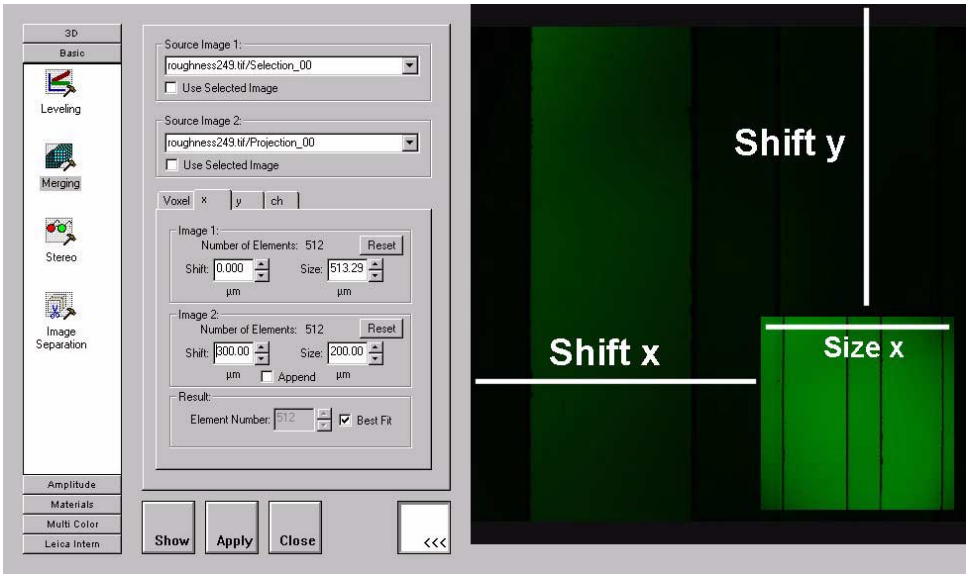


before leveling

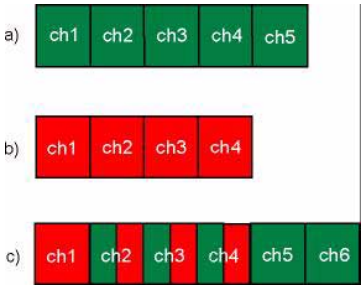


after leveling

Example of moving an image in the result image



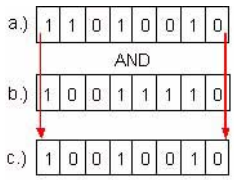
Example of merging images with different numbers of channels



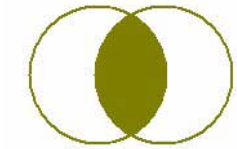
a.) First output image (setting: Start=2)
b.) Second output image (setting: Start=1)
c.) Merged image

How Boolean Operations Work

AND — Write a 1 if there is a 1 in pixel 1 AND in pixel 2; otherwise write 0

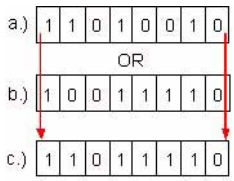


a.) Binary representation of pixel 1
b.) Binary representation of pixel 2
c.) Binary representation of result pixel



AND represents the intersection of two images

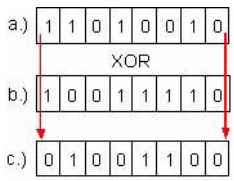
OR — Write a 1 if there is a 1 in pixel 1 OR in pixel 2; otherwise write 0



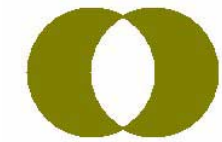
a.) Binary representation of pixel 1
b.) Binary representation of pixel 2
c.) Binary representation of result pixel

OR represents the union of sets of two images

XOR — Write a 1 if the values in pixel 1 and in pixel 2 differ; otherwise write 0



a.) Binary representation of pixel 1
b.) Binary representation of pixel 2
c.) Binary representation of result pixel



XOR represents the union of sets without the intersection of two images.

LCS File Formats

Formats of User-specific and Device-dependent Data

The LCS software provides the following file formats for user-specific data:

	Directory: Leica	Directory: User
Instrument data (**I**nstrument **P**arameter **S**ettings)	*.IPS	*.IPS
Macros	Company.mac	User.mac
Templates for operator console	*.pbo	*.pbo
User profiles	CompanyProfile.pro	PersonalProfile.pro LastExitProfile.pro LastRecent.lst
Templates	*.prt	*.prt
Calibration data	Machine.lhw	
Color Look-Up Tables	*.lut	
User-specific profile as it was defined the last time in use		*.lst

In detail, the different file formats contain:

***.IPS**
Instrument parameters are saved in this format. This includes hardware-based settings such as intensity of the individual laser lines, number of channels used simultaneously, type of main beam splitter used, position and bandwidth of individual spectrophotometer diaphragms and sensitivity of detectors. You can use factory-defined standard sets of instrument parameters that cannot be changed but that allow for a very quick configuration of the hardware. In addition, every user can save instrument parameter sets in a separate user directory. This allows you to save the instrument parameters typical for your planned use so that they can be reproduced and made available for other experiments.

***.mac**
Macros are saved in this format. Macros contain VBA (**V**isual **B**asic for **A**pplication) program code. Leica provides the individual program objects for controlling the confocal system in an object model. Additional information about the development of macros for controlling the confocal system can be found in the chapter on "The LCS Macro Language". The software includes factory-predefined macros that can be used without modifications or modified according to your particular needs. The self-designed or modified macros are stored in the respective user-defined directory.

***.pbo**
Templates for the assignment of the control panel are saved in this format. These templates define which scan parameter is controlled by which rotary knob. This format also includes factory presets that have proven useful for most standard applications. Self-defined templates are saved in the user-specific directory.

***.pro**
Specific profiles of the user interface are saved in this format. This includes information on the type of key and its location in a specific toolbar. In addition, new toolbars can be defined or existing defined toolbars can be hidden or displayed. The position of the toolbars–

whether embedded or freely moveable–is also stored in a profile. This allows every user to save an individual user interface. For example, system administrators can use this tool to create a separate user interface for each knowledge level of the user (beginner, advanced, expert).

***.lst**

Besides the individual user-specific profiles, the user directory also contains the profile of the last session as well as a list of the last four data records applied by the user.

***.prt**

Templates for the "Viewer" window are saved in this format. This allows for specifically saving the appearance and type of displayed information. An explanation of the individual configuration options can be found in the chapter on "Viewer window".

***.lhw**

Calibration data and hardware settings of the confocal system are saved in this format. This file may be edited only by service personnel certified by Leica Microsystems Heidelberg.

Warning

Changes to this file may result in serious, possibly irreparable damages to the confocal system. Damages demonstrably resulting from changes to this file by the user will result in a loss of warranty for the complete system.

***.lut**

Color look-up tables are saved in this format. They are used to assign the intensity values measured by the detectors to each color. This allows to bring out not only the true color display, but also certain intensity areas. More details can be found in the chapter on "Selecting Color Look-Up Tables."

Fixed Leica-specific File Formats

The following file formats can be opened and viewed in the Leica Confocal Software:

Raw data (*.raw)

The RAW format saves data as a linear two-dimensional array in the INTEL format. The column index of the array corresponds to the fastest scan dimension (in general the x-axis), while the line index corresponds to the slower scan dimension (in general the y-axis). In an 8-bit image, each measured variable is saved as a single byte. A 12-bit image uses 2 bytes, whereby the first byte contains the higher-valued bits in accordance with the INTEL format (little endian). Each optical section is saved for each channel in a separate file with the file extension .raw using the following naming convention: *name of experiment_name of data record_channel number_z-dimension_number of optical sections*.raw

Experiments (*.lei)

The format of this type of file is Leica-specific and binary. This format is provided for the data of entire experiments.
A file description is saved in this file format, referencing a series of image files in TIF format or RAW format.

Tiff files (*.tif)

These are Leica image files in single and multi TIFF format. Both image files in the previously used TCS formats and external files in RGB TIF

format can be read.

Annotation (*.ano)

The format of this type of file is also Leica-specific and binary. It is used for saving annotation sheets. The elements on the annotation sheet, such as images, texts and graphic images, are each available as individual objects.

Note

Information on whether the stored measured variable is an intensity value or a height value is saved in the corresponding LEI file.

Specification of the "Lei" file format version: beta 2.000

When a file is saved in the LCS software, a subdirectory is first created under the current directory.
The following files are saved in this subdirectory:
1. A description file– its structure is described below. This file is used for interpreting the data of an experiment through the LCS software.
2. The individual image files – the following convention is used in assigning the name of the image files:
NAME_channel number_dimensionIndex. Thus, Chloroplast_C1_Z1 means that "Chloroplast" is the name of the data record assigned by the user, that the image corresponds to the first optical section of a three-dimensional data stack (Z1), and that the data was acquired in the first detection channel (C1).

Structure of description file

The description file can be read only by the LCS software.
It consists of a collection of various tables and contains the following structure:

Header table

Byte	Meaning	Possible values
0 ... 3	Byte arrangement	0x49494949 Intel LSB 0x4D4D4D4D Motorola MSB
4 ... 7	Identification for Leica "Lei" format	0x016033F0
8 ... 11	Version number	current: =x20000000
12 ... 15	Address of directory table A	any possible address within the complete length of the file

Directory table A

Address	Meaning	Entry data type
Address of A = A	Number of entries in this table	DWORD
A+4	Index of the first entry (represents a logical memory block, e.g., acquisition parameter ("hardware settings") or image parameter ("dimensions"))	DWORD
A+8	Address A1 of the 1st entry	DWORD
A+12	Index of the first entry (represents a logical memory block, e.g., acquisition parameter ("hardware settings") or image parameter ("dimensions"))	DWORD

A+14	Address A2 of the 2nd entry	DWORD
…	...	...

The length of the directory table depends upon the number of images and image data to be saved in an experiment (acquisition parameters, number of channels, number of optical sections, etc.).
Each entry in the directory table corresponds to a logical memory block.

Block table Xn

Address	Meaning	Data type
A1 or A2 or A3.....A_i	Check digit	DWORD
A_i+ 4 bytes	Description of the contents of the block table (currently not used)	DWORD
A_i+ 8 bytes	Version of entry	DWORD
A_i+ 12 bytes	Size of entry	DWORD
A_i+ 16 bytes	Begin of entry	The data type depends upon the entry type.

The following logical memory blocks are available:

const DWORD	ID_SERIES	=	10;
const DWORD	ID_IMAGES	=	15;
const DWORD	ID_DIMDESCR	=	20;
const DWORD	ID_FILTERSET	=	30;
const DWORD	ID_TIMEINFO	=	40;
const DWORD	ID_SCANNERSET	=	50;
const DWORD	ID_EXPERIMENT	=	60;
const DWORD	ID_LUTDESC	=	70;

A memory space of 32 bits is defined for the DWORD data type.

ID_SERIES memory block

This data block contains information about the size of the complete data series combined in an experiment. It always occurs only once per experiment and is structured as follows:

Size [byte]	Data type	Symbol	Description
4	int		Internal version number
4	int	nSE	Number of image series
4	int	nIm	Length of a file name in wchar
4	int	nExt	Length of the file extension of the image file in bytes
nExt	wchar		File extension of image file

ID_IMAGES memory block

This block contains all file names of all image files that are part of a series. The data block is structured as follows:

4	int	nFiles	Number of single images of the series
4	int		Width of a single image
4	int		Length of a single image
4	int		Bits / data points (image resolution)
4	int		Data points / pixels (display resolution)

For the next *n*

image files				
	nIm * 2	wchar		Name of the next image

ID_DIMDESCR memory block

This block provides a description of an image in an n-dimensional space.

	Size [byte]	Data type	Symbol	Description
	4	int		Internal version number of the VOXEL description
	4	int		Voxel type, e.g., RGB or GRAY (see the following VOXEL TYPES table)
	4	DWORD		Byte size of a pixel Typical: (1 or 2) or (3 or 6 for RGB)
	4	DWORD		Resolution of scan data (8, 12 or 16 bit)
	4	int	nTC	Length of the following string in wchar (wchar = **w**ide **char**acter); character format that uses a 2-byte coding of characters unlike the ASCII format (1 byte).
	nTC * 2	wchar		Maximum value of measured variable (intensity value or length value of a distance) for a voxel.
	4	int	nTC	Length of the following string in wchar
	nTC * 2	wchar		Minimum value of measured variable (intensity value or length value of a distance) for a voxel.
	4	int	nTC	Length of the following string in wchar
	nTC * 2	wchar		Designation of measured variable ("I" for intensity, "z" for length value)
	4	int		Internal version number
	4	int	nDims	Dimension of the image, e.g., x_y_ch_z = 4
For the next *n* dimensions				
	4	DWORD		Identification number (ID) of the dimension (see the following list "Identification numbers (ID) for image dimensions")
	4	DWORD		Size of dimension (e.g., 512 pixel)
	4	DWORD		Distance between sub-dimensions (e.g., byte distance between the image series of two consecutive channel numbers)
	4	int	nTC	Length of the following string in wchar
	nTC * 2	wchar		Physical length with unit of length, e.g., 10 µm

4	int	nTC	Length of the following string in wchar
nTC * 2	wchar		Physical start position with unit of length, e.g., 10 µm
4	int	nTC	Length of the following string in bytes
nTC	wchar		Name of the image series
4	int	nTC	Length of the following string in bytes
nTC	wchar		Description of the image series

Identification numbers (ID) for image dimensions
The following identification numbers are possible:

ID (decimal)	Meaning
0	undefined
120	x
121	y
122	z
116	t for time dimension
6815843	channel number
6357100	wavelength Range
7602290	rotation
7798904	x-wide for motorized xy-stage
7798905	y-wide for motorized xy-stage
7798906	z-wide for z-stage
4259957	user1 (not specified)
4325493	user2 (not specified)
4391029	user3 (not specified)
6357095	gray-scale, e.g., of histogram
6422631	gray-scale1, e.g., of histogram
6488167	gray-scale2, e.g., of histogram
6553703	gray-scale3, e.g., of histogram
7864398	logical x (not physical, but logical position value)
7929934	logical y (not physical, but logical position value)
7995470	logical z (not physical, but logical position value)
7602254	logical t (not physical, but logical position value)
7077966	logical wavelength value (not physical, but logical value)
7471182	logical rotation value (not physical, but logical value)
5767246	logical x-wide value (not physical, but logical value)
5832782	logical y-wide value (not physical, but logical value)
5898318	logical z-wide value (not physical, but logical value)

ID_FILTERSET memory block
This memory block describes the hardware settings such as pinhole diameter or selected filters. The memory block starts with a SAFEARRAY header that describes the number and size of the entries. A complete description of the SAFEARRAY structure can be found in Appendix 1. Presently, only three entries of the SAFEARRAY structure are used:

1. sa.cDims should = 1. Presently, only one-dimensional data fields (arrays) are used.
2. sa.cbElements Size of elements in byte
3. sa. rgsabound[0].cElements indicates the number of elements contained in the structure

	Size [byte]	Data type	Symbol	Description
	24	SAFEARRAY	sa	Microsoft type, see Appendix 1
for all elements that contain the structure = sa.rgsabound[0].cElements				
	128	wchar		Identifier for the contents of the entry
	64	wchar		Name of a short description, e.g., a TIFF description tag
	64	wchar		String (contains the contents of the string)
	16	VARIANT		Contains the data type and the data value (except for the "string" data type which is described in the field above)
	4	DWORD		Separate memory space for data values
	4	DWORD		Not in use
	4	DWORD		Identifier (ID) of the memory block (for internal use only)
	4	DWORD		Test value

ID_TIMEINFO memory block
This memory block contains the time taggers for image series that were acquired with a time-dependent scan mode.

Size [byte]	Data type	Symbol	Description
4	int	nDims	Indicates the number of

						dimensions that the description of the time tagger includes
		4	int			Indicates which dimension was provided with a time tagger
For the next *n* dimensions						
		4	DWORD			Identifier (ID) of the dimension (see the description of the D_DIMDESCR memory block)
		4	DWORD			Size of the dimension (number of elements, e.g., 512 pixel)
		4	DWORD			Distance between the individual dimension entries
		4	int	nTS		Number of time taggers
For the next *n*time taggers = nTS						
		64	wchar			Time tagger as string ("string" data type)
		4	int	nTM		Number of time tags per time tagger
For the next *m* time tags = nTM						
			4	int	nC	Number of dimensions of the time tagger description
	loop nC					
			4	int		Coordinate in this dimension
			64	wchar		Time tag as string (wchar data type)

Example:

Image with the following dimensions and dimension sizes:

x	y	ch	z
512	512	3	10

For example, the description of a time tag is 512_512_3_10. This description matches the description of the image. The time tagger description 1024_1024_3_10 for the above sample image means that the original image featured an xy-dimension of 1024*1024 and was reduced by the software (downsampling). In such cases, the time tag must be re-calculated accordingly.

A time tag can be defined at any time during an n-dimensional data acquisition process (scan). The coordinate of the time tag 0_0_2_7 indicates that the time tag was set at the start of a data record (0_0_c_#) on the second channel (0_0_2_#) for the 7th optical section (0_0_2_7).

ID_SCANNERSET memory block

This memory block contains device parameters that are set at the time of the image acquisition.
Further details about the SAFEARRAY structure can be found in the description of the ID_FILTERSET memory block.

Size [byte]	Data type	Symbol	Description
24	SAFEARRAY	sa	Microsoft type, see Appendix for all elements that contain the structure = sa.rgsabound[0].cElements
128	wchar		Identifier for the contents of this entry
64	wchar		Name of a shorthand notation, e.g., a TIFF description tag
64	wchar		String (contains the contents of the string)
20	VARIANT		Contains the data type and the data value (except for the "string" data type which is described in the field above)
4	DWORD		Separate memory space for data values
4	DWORD		Not in use
4	DWORD		Identifier (ID) of the memory block (for internal use only)
4	DWORD		Test value

ID_EXPERIMENT memory block

Description of the used save format (e.g., “*.lei file with PC-Tiff images”)

Size [byte]	Data type	Symbol	Description
4	int		Internal version number
4	int		Number of images in the data collection of the experiment
4	int	nTC	Length of the following string in wchar
nTC * 2	wchar		Short description of the format
4	int	nTC	Length of the following string in wchar
nTC * 2	wchar		File extension of the main file
4	int	nTC	Length of the following string in wchar
nTC * 2	wchar		Identifier of the single image format (e.g., PC-TIFF)
4	int	nTC	Length of the following string in wchar
nTC * 2	wchar		File extension for the single image format (e.g., tif / raw)

ID_LUTDESC memory block

Description of the color look-up tables (LUT's)

	Size [byte]	Data type	Symbol	Description
	4	int	nLU	Number of channels
	4	DWORD		Identifier (ID) of the dimension whose display is being colored.
For the next channels until = nLU				
	4	int		Internal version number
	1	bool		bool IsInverted
	4	int	nTC	Length of the following string in bytes
	nTC	wchar		Description of the color look-up table (LUT)
	4	int	nTC	Length of the following string in bytes
	nTC	wchar		File name of the color look-up table (if available)
	4	int	nTC	Length of the following string in bytes
	nTC	wchar		Name of the color look-up table
	4	int		Field for internal use
	4	int		Dimension of the color look-up table

Appendix 1

SAFEARRAY Data Type

```
typedef struct FARSTRUCT tagSAFEARRAY {
    unsigned short cDims;         // Count of
dimensions in this array
    unsigned short fFeatures;     // DON'T CARE
    unsigned long cbElements;     // Size of an element
of the array.
                                  // Does not include
size of
                                  // pointed-to data.
    unsigned long cLocks;         // DON'T CARE
    void HUGEP* pvData;           // Pointer to the
data.
    SAFEARRAYBOUND rgsabound[1]; // One bound for each
dimension.
} SAFEARRAY;
```

Important: The Leica-specific file format "LEI" uses only one-dimensional data fields (arrays).

SAFEARRAYBOUND Structure
Represents the bounds of one dimension of the array. The lower bound

of the dimension is represented by `lLbound`, and `cElements` represents the number of elements in the dimension. The structure is defined as follows:

```
typedef struct tagSAFEARRAYBOUND {
    unsigned long cElements;           // num of
elements
    long lLbound;                 // DON'T CARE
} SAFEARRAYBOUND;
```

VARIANT and VARIANTARG

```
typedef struct FARSTRUCT tagVARIANT VARIANT;

typedef struct tagVARIANT  {
    VARTYPE vt;
    unsigned short wReserved1;
    unsigned short wReserved2;
    unsigned short wReserved3;
    union {
        unsigned char      bVal;            // VT_UI1.
        short              iVal;            // VT_I2
.
        long               lVal;            // VT_I4
.
        float              fltVal;          // VT_R4
.
        double             dblVal;          // VT_R8
.
        VARIANT_BOOL       boolVal;         //
VT_BOOL.
        SCODE              scode            //
VT_ERROR.
        CY                 cyVal;           // VT_CY
.
        DATE               date;            //
VT_DATE.
        BSTR               bstrVal;         //
VT_BSTR.
        IUnknown           FAR* punkVal;    //
VT_UNKNOWN.
        IDispatch          FAR* pdispVal;   //
VT_DISPATCH.
        SAFEARRAY          FAR* parray;     //
VT_ARRAY|*.
        unsigned char      FAR* pbVal;      //
VT_BYREF|VT_UI1.
        short              FAR* piVal;      //
VT_BYREF|VT_I2.
        long               FAR* plVal;      //
VT_BYREF|VT_I4.
        float              FAR* pfltVal;    //
VT_BYREF|VT_R4.
        double             FAR* pdblVal;    //
VT_BYREF|VT_R8.
        VARIANT_BOOL       FAR* pboolVal;   //
VT_BYREF|VT_BOOL.
        SCODE              FAR* pscode;     //
VT_BYREF|VT_ERROR.
```

```
        CY                  FAR* pcyVal;     //
VT_BYREF|VT_CY.
        DATE                FAR* pdate;      //
VT_BYREF|VT_DATE.
        BSTR                FAR* pbstrVal;   //
VT_BYREF|VT_BSTR.
        IUnknown FAR*       FAR* ppunkVal;   //
VT_BYREF|VT_UNKNOWN.
        IDispatch FAR*      FAR* ppdispVal;  //
VT_BYREF|VT_DISPATCH.
        SAFEARRAY FAR*      FAR* pparray;    //
VT_ARRAY|*.
        VARIANT             FAR* pvarVal;    //
VT_BYREF|VT_VARIANT.
        void                FAR* byref;      // Generic
ByRef.
     };
};
```

Important: Files with the "LEI" format never use pointers as parameters.

VARTYPE

```
typedef unsigned short VARTYPE;
enum VARENUM{
    VT_EMPTY      = 0,          // Not specified.
    VT_NULL       = 1,          // Null.
    VT_I2         = 2,          // 2-byte signed
int.
    VT_I4         = 3,          // 4-byte signed
int.
    VT_R4         = 4,          // 4-byte real.
    VT_R8         = 5,          // 8-byte real.
    VT_CY         = 6,          // Currency.
    VT_DATE       = 7,          // Date.
    VT_BSTR       = 8,          // Binary string.
    VT_DISPATCH   = 9,          // IDispatch
    VT_ERROR      = 10,         // Scodes.
    VT_BOOL       = 11,         // Boolean; True=-1,
False=0.
    VT_VARIANT    = 12,         // VARIANT FAR*.
    VT_UNKNOWN    = 13,         // IUnknown FAR*.
    VT_UI1        = 17,         // Unsigned char.

    // Other constants that are not valid in VARIANTs
omitted here.

};
    VT_RESERVED    = (int) 0x8000
    // By reference, a pointer to the data is passed.
    VT_BYREF      = (int) 0x4000
    VT_ARRAY     = (int) 0x2000    // A safe array of
the data is passed.
```

The vt value determines the interpretation of the data of the experiment as follows:

Value	Description

VT_EMPTY	No value was entered
VT_UI1	An unnamed 1-byte character was saved in *bVal*.
VT_I2	A 2-byte integer value was saved in *iVal*.
VT_I4	A 4-byte integer value was saved in *lVal*.
VT_R4	An IEEE 4-byte real value was saved in *fltVal*.
VT_R8	An IEEE 8-byte real value was saved in *dblVal*.
VT_CY	A fixed point value is indicated. It consists of 15 digits before the point and 4 digits after the point. The value was saved in *cyVal*.
VT_BSTR	Exceptions for files in LEI format: A string was saved in the variable TCHAR
VT_NULL	A floating zero value was indicated. Here, the floating zero is not a NULL pointer. This value is required for a 3-state logic, for example, in the SQL database query language.
VT_ERROR	An SCODE error was indicated. The error type is specified *scodee*.
VT_BOOL	A Boolean value (true / false) was indicated. The value 0xFFFF (all bits=1) means "true," the value 0 (all bits=0) means "false."
VT_DATE	A value corresponding to a date was indicated. A date is saved as a number in double-precision format. All data is saved as differential dates in reference to January 1, 1900. For example, the entry "25" corresponds to 01-25-1900.

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